THE
AMERICAN NATURALIST

VOL. LXIV *January–February, 1930* NO. 690

INHERITANCE OF DISEASE RESISTANCE IN ANIMALS[1]

PROFESSOR L. J. COLE
UNIVERSITY OF WISCONSIN

THE trend a discussion of inheritance of disease resistance in animals is likely to take will naturally depend to a large extent on what definition of disease is adopted. Huxley defined disease as "a perturbation of the normal activities of a living body." Perhaps one of the dictionary definitions (Century), which explains disease as any "deviation from the healthy or normal condition of any of the functions or tissues of the body," will answer our purpose as well as any. It is true that one may still ask how we are to be sure just what is a healthy or normal condition, but we can save ourselves considerable mental effort and worry if we forego attempting to make water-tight definitions of these words and leave them rather to common usage and common sense.

TWO CLASSES OF DISEASES

There are two large categories of disease depending on whether or not an invading organism is involved. In a general way, diseases may be classed as infectious and non-infectious, though the distinction is by no means clear cut. When the term "disease resistance" is used the implication is that it relates to the infectious diseases and refers to the ability of the exposed individual to ward off or otherwise combat the invading organism or its

[1] Paper from the department of genetics, Agricultural Experiment Station, University of Wisconsin, No. 105. Published with the approval of the director of the station.

103747

effects. Resistance to non-infectious diseases is apt to be of quite a different sort. Nevertheless, from the standpoint of inheritance the latter are of peculiar interest and importance and for that reason seem deserving of consideration in the present connection.

NON-INFECTIOUS DISEASES

Non-infectious disturbances of function are commonly hereditary, and probably in most cases could be shown to be related to some hereditary background. Thus, if a man receives a blow on the head, the extent to which it will disturb his normal functions will depend, among other things, on the thickness and hardness of his skull. These qualities of the bone may be conditioned to some extent by the man's prenatal and postnatal nutrition and other extraneous conditions, but heredity is also an important factor. To that extent, then, we might study the inheritance of the differences in resistance offered by various skulls to the disturbing effects of cranial concussions, but such an investigation is obviously outside the scope of the present discussion.

When we turn, however, to such conditions as Huntington's chorea of man, inherited as a Mendelian dominant and characterized by nervous degeneration and concurrent symptoms in adult life, or to the recessive tumor occurring in the larva of Drosophila, as described by Miss Stark, we have examples of disturbances of normal structure and function which deserve a moment's consideration. What would heritable resistance mean in these cases? Perhaps in the case of the larval tumor the dominant allelomorphs may be considered as heritable factors which contribute something to the tissues of the developing larva, making them resistant to the tumorous growth. Similarly, the condition of the nervous system developing under the influence of the recessive allelomorph of the gene causing Huntington's chorea may possibly be looked upon as a type of resistance to the disease. The case would be more convincing, how-

ever, if a second gene were found which was a direct inhibitor, at least in some degree, of the gene responsible for the disease. Frequent as the occurrence of inhibiting genes is, such a relationship would not be surprising.

Whether any particular heritable condition is detrimental to health or prejudicial to life depends often on conditions. Albinism of our laboratory rat or guinea-pig, for example, would constitute a serious handicap under natural conditions and would doubtless tend to cause a "deviation from the healthy or normal condition" of the individual, if only that it resulted in its being more readily picked up by some beast of prey. In the same way *any* condition which results in lowering the vital index of an animal under any given set of conditions may be looked upon as a "disease" in the broadest sense. This is, of course, stretching to the extreme and much beyond the ordinary usage the concept of disease, but my purpose is to show that no hard and fast line can be drawn and that what may be detrimental to an individual under certain conditions may be negligible as a harmful factor in a different environment. Of non-infectious diseases, therefore, we may have a complete series from those causing profound disturbance and inevitable death, to others which may be only mildly prejudicial to existence under special circumstances. This series would range from those inevitable lethals which bring death in the earliest embryonic stages, through lethals acting in later fetal development or at various ages of postnatal life, through "physiological defectives" of all grades, and would finally include in least degree the bulk of all mutations from the type of the wild species. The differences would appear to be in degree and not in kind.

Resistance to Infections

That there exist heritable differences in resistance of animals (including man) to infectious diseases is too well known to require demonstration. The varying susceptibility of various races of mankind to such diseases as

measles, smallpox and tuberculosis, or the resistance of Brahman cattle to Texas fever, as compared to ordinary cattle, can not be attributed to environment, and hence they constitute sufficient proof of some inherited difference in the men or cattle of the different races. Medical literature, both human and veterinary, is replete with similar indications of particular diatheses or of unusual resistance to infections. While questions of acquired immunity enter in to complicate the situation, there is still sufficient evidence of a hereditary basis in a host of cases. Nevertheless, in few if any diseases has the exact mode of inheritance of the resistance and susceptibility been unraveled. This is due to the many complications and difficulties which beset the investigation of this subject. Some of these may be briefly discussed, and this can perhaps best be done by considering the requisites, or at least the desirable conditions, for successful experimentation in this field.

In the first place, it is desirable to use as the experimental animal one which can be easily and rapidly bred and readily kept in sufficient numbers. Its individual value should be relatively low so that specimens may be sacrificed for experimental purposes without involving too great an expense. This factor, unfortunately, practically rules out the larger domesticated animals for theoretical investigations.

The second desideratum is a disease with symptoms which are readily recognizable, or which can be identified by some other convenient test. Furthermore, it should be of such a nature that uniformity of exposure, or opportunity for infection, may be assured and that a measure of its development in, and effect on, the host may be obtained. Diseases whose causative organisms may be grown in laboratory cultures and standardized as to virulence are naturally best adapted for this purpose. This question of virulence of the disease and susceptibility of the host is apt, however, to lead to a vicious circle in the attempt at the same time to use the host animal for the

purpose of standardizing the virus and to employ the virus as a test of the variation in susceptibility in the host species. It violates the principle that in experimental work only one variable should be investigated at a time, the others being held constant. The use of cultures from single cell isolations, when possible, may help to standardize the virus, though it can not be assumed without occasional check that derivatives of such cultures will be uniform in virulence.

If there were available strains of the host animal inbred until they were presumably homozygous in hereditary factors for disease resistance, they would not only be valuable for the purpose of standardizing the infective organism, but would also greatly facilitate the study of any heritable differences that might exist in the various host lines. Fortunately, in guinea-pigs, rats and mice, a few inbred lines have been produced and are being maintained in a number of laboratories. Such stocks are bound to prove invaluable for genetic studies, including the investigation of disease resistance.

Certain other conditions must be borne in mind. Thus, if the disease is one which kills the host or incapacitates it for future breeding, the young animals for continuing the experiment must be produced before the disease test is made. The possibility of immunity acquired by chance infection or, in the case of mammals, of immunity transmitted from the mother through the placenta, must also be guarded against. Furthermore, while the probability will be considered remote by most geneticists, the possibility of an acquired hereditary immunity (if we may use this term) being built up in a Neo-Lamarckian sense must be kept in mind in planning one's experiment, if only to guard against criticism. This would be presumed to come about through the exposure of the germ-plasm of successive generations directly to substances in the blood which result from reaction of the tissues to the invading organism.

As in most experimental work, it is seldom that ideal conditions are obtainable. Particularly is this likely to be the case when the host or the disease being investigated is determined by practical considerations. Simply by way of illustrating some of the conditions and difficulties which are likely to be met with I may perhaps be pardoned if I describe briefly an investigation which has been under way at the Wisconsin Experiment Station for several years past. This is a study of the inheritance of susceptibility and resistance to contagious or infectious abortion, a disease which causes enormous economic losses in cattle breeding. The causative agent is a bacillus isolated a good many years ago by the Danish scientist Bang, and is commonly known as *Brucella* (*Bacillus*) *abortus,* or simply as "Bang's bacillus."

It should first be mentioned that the investigation is a cooperative one between the departments of genetics and veterinary science, the two departments being responsible respectively for the study of the heredity and pathology of the disease.[2] This is an important point, and should be emphasized, for it is almost futile to attempt to study the inheritance of disease resistance, either in animals or plants, without a correlative study of the pathology.

The difficulties of attempting to work out the inheritance of resistance to contagious abortion directly with cattle are too evident to need comment. We have chosen to use rabbits as the host species, since they meet the requirements of a small laboratory animal and it was found that they reacted much as cattle do to a particular strain of the abortus organism; that is to say, when pregnant females are given intraperitoneally at the sixteenth day of pregnancy a standard inoculation of the *B. abortus* culture, most individuals abort their fetuses before birth,

[2] Cooperative support of this investigation is also being given by the Bureau of Animal Industry, U. S. Department of Agriculture. The work here reported has been done largely by Dr. B. L. Warwick and Dr. Miguel Manresa.

while a few carry through and give birth to living young at full term. This fact was taken advantage of as a basis of selection, and to make a long story short, by close breeding and selection pedigree lines have been produced which differ widely in their response to the inoculation. One group is practically 100 per cent. susceptible under the conditions of the experiment, while the other shows a resistance of about 85 per cent. The most interesting genetic indication is that susceptibility appears to be clearly a recessive. Susceptible mated to susceptible produces only susceptible. Furthermore, there is some indication that only a single pair of genes is concerned. If such is the case, it may be asked why it has not been possible to isolate a line homozygous for the dominant gene, which would consequently breed true for resistance. One difficulty is that we are dealing with a disease the effects of which can be measured, comparably at least, only in the female. There is another interesting situation which may also be a factor. This involves the possible interrelation of fetus and dam. We ordinarily think of the resistance to abortion as being a characteristic exclusively of the maternal organism. If we assume for the sake of simplicity that heritable resistance and susceptibility do in fact depend on a single pair of Mendelian genes, it is evident that the genetic relationship of the dam and the fetuses she may be carrying will depend on her own genotype and that of the male to which she is bred. In a susceptible (recessive) doe bred to a susceptible buck all the fetuses as well as the maternal tissues will be genetically susceptible, and uniformity of abortion is to be expected if the female is inoculated with the appropriate dosage of the virus at the proper time. This is the result obtained. But what may we expect if the susceptible doe is bred to a homozygous resistant buck? In this case, while the mother is susceptible, the fetuses themselves, though heterozygous, are genetically resistant. The experimental results so far obtained indicate that the presumed genetic resistance of the fetuses is not sufficient to pro-

tect them from premature expulsion when the maternal tissues, and particularly the maternal placenta, are favorable to the infection.

The situation is somewhat different when resistant does are considered. If the doe is homozygous for resistance, her young should be resistant whatever the sire, and no abortion would be expected. We have not yet with certainty found such a female, but some of the difficulties of doing so have already been touched on.

In the case of a heterozygous resistant doe the situation is more complex. Bred to a homozygous resistant buck, uniformity of resistance is to be expected. The few bucks that have been adequately tested, however, have all given indication of being heterozygous. Now if the heterozygous doe is bred to a heterozygous resistant or to a susceptible buck a portion of her fetuses will be genetically susceptible—one fourth on the average in the former case and one half in the latter. Here we have both resistant and susceptible fetuses in a resistant body. The question is, can the invading organism under these circumstances survive long enough in the maternal tissues and pass through the placentas so as to infect the susceptible fetuses? It seems possible that this may occur and that it is an explanation of what appears as an intermediate resistance in the case of certain supposedly resistant does which carry their fetuses to full term but then give birth to still-born as well as living young. Further weight is lent to this explanation by the recovery of the organism from such dead fetuses, while it is seldom or never recoverable from the normal living young.

The foregoing explanation must be considered as still in the stage of a working hypothesis, but it brings us squarely up against the question as to what resistance really is. Is it a toleration of the tissues to infection—the ability to grow and function normally in spite of the presence of the disease germ—or is there an actual combating of the organism and an attempt to eliminate it from the body? These are really questions for the im-

munologist and the pathologist, but I think enough has been said to show how intimately they are associated with the problems of genetics and how important it is that one branch of science should serve the other.

Practical Considerations

Whether it is more practicable from an economic standpoint to establish disease-resistant strains or to control disease through other measures is a matter which must be worked out in each particular case. It depends on a great number of factors, involving all sorts of conditions, such as the nature of the disease and its etiology, the manner of propagation of the host species, its individual value and the interaction of host and parasite to all the conditions of the environment. The plant breeder has been able to demonstrate beyond question the value and economic importance of disease-resistant plants, and the use of the breeding method to combat plant diseases seems to be steadily increasing. Considering, however, the limitations of reproduction in the larger domesticated animals, the relatively great value of the individual, the inflexibility of breeding methods imposed by breed regulations and the doubtful practicability of intensive inbreeding, many are frankly skeptical as to the practical possibility of utilizing heritable resistance as a considerable factor in live-stock production. Even though resistance to contagious abortion should prove to be inherited as a simple Mendelian dominant in cattle, any geneticist familiar with cattle breeding would see at once the difficulties attending the establishment of a homozygous resistant strain of dairy cattle which could compete also in other respects with the best existing herds. It seems quite probable, however, as indicated by the selection experiment of Roberts for resistance to bacillary white diarrhea in chickens, that the practical application of disease resistance in fowls may be much more easily accomplished. Correspondingly, work with swine and

sheep should yield results more rapidly than with cattle or horses.

Perhaps the most immediate practical value of the establishment of strains of both laboratory and larger animals which are homozygous and consequently genetically uniform in their reaction to specific diseases lies in their usefulness to students of pathology, immunology and nutrition. An experiment is under way at the present time at Wisconsin to test the effect of the plane of nutrition of dairy cattle on their reaction to abortion infection. The numbers used must necessarily be small, and there is at present no way of knowing to what extent genetic diversity exists in the experimental animals and how it may affect the results. If stock known to be genetically homogeneous could be selected for such an experiment the difficulties of obtaining significant and comparable results would be greatly reduced. Enough has already been said in connection with the rabbit experiment to show that a knowledge of the genetics should there make possible a line of pathological investigation that would otherwise be practically unapproachable.

Though the foregoing discussion may have seemed to stress the difficulties of investigating disease resistance in animals and of applying the results, I hope it will not leave an impression of discouragement and pessimism. The encouraging thing is that differences in resistance and susceptibility in animals may clearly be heritable, and hence they offer suitable material for genetic investigation and for possible practicable application. The difficulties encountered constitute a challenge to the ingenuity and patience of the investigator, but are by no means insurmountable. It is to be hoped that such information as we now have will lead to more concerted attacks on the problems of disease resistance by workers in all the related fields. There is every reason to believe that such endeavors will gradually be rewarded by results of both theoretical and practical importance.

INHERITANCE OF DISEASE RESISTANCE IN PLANTS[1]

PROFESSOR H. K. HAYES

UNIVERSITY OF MINNESOTA

THE application of genetic principles to the production of improved varieties of crop plants has placed the work of the plant breeder on a sound, scientific basis. In connection with the production of new varieties by hybridization, Pearl has stated, "The plant breeder can and does make Mendelism his direct and immediate guide. He has made Mendelism the working tool of his craft."

The value of disease resistance in plants is universally recognized, and important results both of a scientific and practical nature have been obtained by the application of the laws of genetics to the production of disease-resistant varieties. The literature relating to plant breeding and plant pathology, in recent years, contains numerous papers recording the studies which have been made on disease resistance. In almost all carefully outlined plant-breeding projects, plans are made to obtain varieties resistant to the more destructive diseases.

In most breeding problems, it is essential to grow the experimental crop under normal environmental conditions for the purpose of isolating the better sorts. In breeding for resistance to disease, the organism which causes the disease must receive as much attention as the crop itself, and some means of obtaining an epidemic of the disease must be found. Besides the genetic factors for resistance or susceptibility carried in the host plant, there may be genetic factors in the pathogene which must be considered also. Because of the nature of the problem, there is an unusual opportunity for a cooperative

[1] Published with the approval of the director of the Minnesota Agricultural Experiment Station, as paper No. 826 of the Journal Series of the Minnesota Station. Read at a joint session of the Genetics Sections with Geneticists Interested in Agriculture, December 27, 1928.

attack with the plant geneticist and plant pathologist in the major rôles. Such cooperative studies have been under way at Minnesota for slightly more than ten years.

In a discussion of the subject "Inheritance of Disease Resistance in Plants," it appears logical to present the matter under four general heads: First, the economic importance of disease resistance; second, a genetic analysis of the nature of pathogenic organisms; third, the genetics of disease resistance in plants, and fourth, the nature of resistance. As there are so many data available regarding certain of these phases, it seems best to outline briefly under each of these four headings the present status of studies of disease resistance in plants without any attempt to make an exhaustive summary.

Economic Importance of Disease Resistance

The serious losses from diseases caused by pathogenic organisms can be appreciated by referring to a statement in the *Plant Disease Reporter* of the Bureau of Plant Industry. A summary made in 1926 recorded the estimated yearly losses from diseases for twelve crop plants for the period from 1919 to 1925 inclusive. These data were obtained from estimates submitted by disease specialists throughout the United States. No claim is made that the figures are accurate, but they are believed to be the best available. The range of yearly variability is given here, together with the average loss for the seven-year period for seven important farm crops.[2]

Crop	Range of estimated losses	Average
Wheat	8.9–17.0	10.8
Rye	1.4– 2.3	1.9
Barley	3.8–11.2	5.0
Oats	4.8– 6.8	6.4
Corn	6.0–10.7	8.0
Potatoes	16.2–21.7	19.6

[2] *Plant Disease Reporter*, Bureau of Plant Industry, U. S. D. A., 1926.

Experimental studies to determine the importance of disease resistance are not necessary in some cases because the disease may be the limiting factor in the production of the crop. Wilt in flax has often proved the limiting factor in the successful growing of flax in the northwest spring wheat area. With the production, introduction and use of resistant varieties by the North Dakota and Minnesota Agricultural Experimental Stations, the farmer can now grow flax with the assurance that wilt will not destroy the crop. Resistance to wilt, however, is only relative and, in order to receive good yields of flax when grown on "wilt sick" soil, it is necessary to sow early (Barker, 1923). Cabbage yellows is another disease which has limited the production of the crop (Jones *et al.*, 1915, 1920). Resistant varieties have been selected, by the Wisconsin station and by the U. S. Department of Agriculture, which produce satisfactory crops when grown on infected soil.

Stem rust in wheat in 1919 was the chief factor in reducing yields in the spring wheat area of the United States (Hayes and Stakman, 1922). The estimated loss from disease for all the United States was 17 per cent. Other years, in relatively recent times, in which there were severe epidemics were 1916 and 1920. In the three years, 1916, 1919 and 1920, the average yield of 8.6 bushels per acre was obtained. In seven other years during the ten-year period from 1912 to 1920, when stem rust was not so serious a factor, an average yield of 15.4 bushels was obtained. In seasons of state-wide or regional epidemics, comparisons of this nature emphasize the losses from diseases and the need for disease-resistant varieties. No agronomically satisfactory spring wheats of the *Triticum vulgare* group resistant to stem rust have been available until recently. Minturki winter wheat, however, is resistant to stem rust under field conditions, and has now been grown in southern Minnesota for ten years. In seasons when other stem rust susceptible winter wheats have been severely injured by rust, Minturki has given good yields.

Estimated losses indicate the relative importance of various diseases. It is recognized by all that experimental studies are needed to determine the actual losses from diseases under various conditions. The breeder can proceed with greater assurance toward the goal of crop improvement, by the production and use of improved varieties, if an accurate knowledge is available of the relative importance of the various characters of the crop in question.

Several methods have been tried to determine the losses from particular diseases. Garber (1922), in studies of stem rust, obtained the comparative yields of resistant and susceptible oat plants in the F_2 generation of crosses between resistant and susceptible parents. In this case, he was dealing with open and side panicles as well, and the crosses were grown in an artificially produced epidemic. The comparative results were as follows:

Type of plant	Average yield per plant
Open panicled, resistant	2.06 gms
" " susceptible	1.54 "
Side " resistant	1.99 "
" " susceptible	1.42 "

The average reduction in yield as a result of stem rust infection was approximately 27 per cent.

Such a method gives accurate results when the genetic factors for resistance and susceptibility are not linked in inheritance with factors for yield. As only a single factor pair was involved for resistance and susceptibility, it appears probable that the differences obtained were a result of the manner of reaction to the disease.

In recent years, the importance of resistance to disease has been determined by growing varieties under comparable conditions and correlating the yields with other characters, including reaction to diseases. Methods of

this nature can be used only when resistant, partially resistant and susceptible varieties are available. Partial and multiple correlations were used in analyzing the results (see Goulden and Elders, 1926; Hayes *et al.*, 1927; Immer and Stevenson, 1928). Studies conducted in Minnesota with spring and winter wheats and with oats have given added knowledge of the relative importance of agronomic characters and disease resistance. By means of a biometrical analysis, it was learned that stem rust of wheat caused severe shriveling of the grain, while reaction to leaf rust was not correlated with grain plumpness. Nevertheless, leaf rust was as strongly correlated with yield when other factors were held constant as was reaction to stem rust. Data on rust reaction are taken on the percentage basis. On the basis of the partial correlation studies with 280 strains of oats and by the use of the partial regression relationship, the statement was made "that on the average an increase of 1 per cent. in crown rust infection resulted in a reduction in yield of 0.32 bushels per acre." Some lines were more severely infected than others. The yields of the most susceptible lines were reduced approximately twenty bushels per acre by crown rust when compared with the most resistant lines.

Perhaps more is known regarding resistance to diseases in wheat than in any other crop plant. At any rate, the writer has a greater first-hand knowledge of disease resistance in spring wheat than in other crop plants. Cooperative studies in Minnesota by plant geneticists and plant pathologists of the Minnesota Agricultural Experiment Station and agronomists and pathologists of the U. S. Department of Agriculture have been carried on since 1915, and a project to produce rust-resistant spring wheats by breeding was outlined by Dean E. M. Freeman, of Minnesota, in 1908.

Among the more serious diseases of wheat are stem rust, leaf rust, scab, bunt, loose smut and black chaff.

Reaction to all these diseases has been studied in recent years in Minnesota. The importance and possibilities of disease resistance can be emphasized by the studies conducted in the spring wheat belt, without attempting a summary of experiments in other states or throughout the world.

Fairly satisfactory stem rust resistant wheats have been obtained in Minnesota and North Dakota. Ceres, a selection from a cross of Kota × Marquis, produced by Waldron, of North Dakota, has been grown commercially for several years. It gives good yields and is satisfactory in milling and baking quality. It is somewhat susceptible to leaf rust, bunt, loose smut and black chaff. Marquillo, the Minnesota production, was obtained from a cross of the durum wheat Iumillo with Marquis (Hayes *et al.*, 1920, 1925). It was increased this year, and approximately 1,400 bushels are available for the first distribution to farmers. So far as known, it is the first instance of the production of a promising commercial variety from a cross of durum and common wheats. It is resistant to stem rust, somewhat resistant to leaf rust, black chaff and bunt, but somewhat more susceptible to root rots than some other varieties, although not markedly inferior in this respect to such wheats as Quality and Marquis. It mills and bakes satisfactorily, as determined from preliminary trials, although it is somewhat inferior in color of leaf.

The only known means of controlling scab is by the use of resistant varieties. Preston and Haynes Bluestem wheats are resistant to scab when grown in the spring wheat area, while Marquis is rather susceptible. Marquis, however, in the spring wheat area, is fairly resistant to bunt and loose smut. Many of the new hybrids under test at present are resistant to scab. Several selections produced by McFadden in South Dakota from crosses of Emmer with Marquis have the chromosome numbers of common wheat and are highly resistant to

stem rust, leaf rust, bunt and loose smut. They are susceptible, however, to black chaff.

These illustrations indicate that, in spring wheat, there is no lack of resistance to most of the important diseases. It should be recognized that acquired immunity in plants, while possible (see Kostoff, 1928), is probably of no importance to the breeder; all that he can do is to isolate resistant strains when they are present in nature and combine the character with other desirable characters by breeding methods. After obtaining resistance in different varieties to all diseases, there remains the problem of combining these resistances in a single variety. Considerable progress has been obtained with spring wheat. Reselections from Kota × Marquis crosses have been made by Waldron at North Dakota which are somewhat superior to Ceres in resistance to stem rust and which, in addition, have resistance to leaf rust and some other diseases. Recrosses of Marquillo with other wheats, particularly homozygous spring wheats selected from Kanred-Marquis crosses, have been studied at Minnesota. Homozygous selections have been obtained and yield trials in four localities have been made for the last two years. These selections have the resistance to stem rust of Marquillo, the immunity to certain physiologic forms of stem rust of Kanred, resistance to bunt, scab and loose smut as well as resistance to root rots, the latter character probably being obtained from Kanred. Several of these selections have yielded 20 to 30 per cent. more than Marquillo on the average, and during these two years Marquillo has yielded more than Marquis.

It is admitted that the problem of obtaining varieties resistant to all important diseases, together with satisfactory agronomic characters, is a difficult one. As evidence of their belief that such studies are worth while, investigators of the four spring wheat states, North Dakota, South Dakota, Minnesota and Montana, and the Bureau of Plant Industry of the U. S. Department of

Agriculture have joined in a cooperative project to obtain satisfactory disease-resistant varieties of spring wheat for this area.

Accepting the fact that the production and use of disease-resistant varieties is worth while and the most satisfactory method of controlling pathogenic organisms when disease-resistant varieties can be obtained, we may next consider the present view-point regarding the genetic nature of disease organisms.

A GENETIC ANALYSIS OF THE NATURE OF PATHOGENIC ORGANISMS

At one time, there was a belief that pathogenic organisms were unstable entities and had the power of gradual adaptation. One method which pathologists thought might change the parasitic capabilities of a fungus was its culture on an intermediary host plant. Evans (1911), studying stem rust of wheat, stated that the hybrid between a susceptible and immune variety of wheat changed the rust to such an extent as to enable it not only to attack the susceptible variety more severely but even to infect the immune parent.

Stakman and others (1918) have made careful studies of this question with *Puccinia graminis* and were unable to corroborate Evans' results. They obtained no evidence that the pathogenic capabilities of a rust form could be changed by association with a particular host plant. It was a matter of common knowledge that varieties which were susceptible in one locality or season were severely injured in some other locality or season by the same disease. The fact that there are physiologic forms of pathogenic fungi explains logically these variations in reaction in different crop seasons.

It is now a generally accepted fact that morphologic species of fungi contain definite entities which can be differentiated only by their manner of reaction on strains of crop plants. Stakman (1926–27) summarized the development of the present view-point of racial specializa-

tion in plant disease fungi. Free use has been made of this publication in this brief review. According to Stakman, there are three principal methods by which physiologic forms can be recognized. These are: (1) by their reaction on selected plants, (2) by cultural characters when grown on artificial media and (3) by physicochemical reactions. In certain cases, also, there are slight morphological differences between physiologic forms.

Physiologic forms are considered to be definite genetic entities, and they are believed to be as constant as many species of higher plants.

Physiologic specialization is common in widely separated groups of fungi, including the rusts, the smuts, the powdery mildews and the root and stem rots of small grains. Extensive studies have been made of the number of physiologic forms in the rust fungi. In stem rust of wheat, over fifty physiologic forms have been isolated, and six physiologic forms have been demonstrated for stem rust of oats. It is obvious that the student of disease resistance in plants must recognize the fact that there may be many genotypic strains of the organism which cause a particular plant disease and that these strains must be used in attempting to obtain resistant varieties.

The importance of physiologic forms may be illustrated by recent studies in Canada (Gordon and Bailey, 1928) of physiologic forms of stem rust in oats. These studies may be used also to present the method of isolating physiological forms of a disease by their reaction on varieties of crop plants. These studies were conducted by inoculating seedlings of four varieties of oats with rust collections. Without using much detail, it may be said that extreme resistance with the production of very small uredinia was classified under group 1, that a slightly lower degree of resistance was classed as 2, that resistance of the White Russian type was called 3 and

that complete susceptibility was classed in group 4. It may be pointed out that resistance of grade 3 in the seedling state was correlated with a very high degree of resistance under field conditions where the disease commonly appeared at about heading time. In some cases, a single physiologic form of a disease uniformly produced both large pustules and small ones on the same leaf or plant. Such a type of infection is classed under group x.

Reaction of the six physiologic forms of stem rust to four varieties of oats used as differentials is given in the table.

Oat variety	Form 1	Form 2	Form 3	Form 4	Form 5	Form 6
Victory	4	4	4	4	4	4
Joanette, Strain 703	1	4	1	1	x	4
Richland	2	2	2	4	2	4
White Russian	3	3	4	4	3	4

It will be noted that Victory is susceptible to all six forms; White Russian is resistant to forms 1, 2 and 5, but susceptible to 3, 4 and 6; Richland is resistant to forms 1, 2, 3 and 5, but susceptible to 4 and 6, while Joanette is resistant to all but forms 2 and 6. At present, there are no varieties known which are resistant to form 6. Studies of the relative frequency of these six forms have been made from 1925 to 1927, inclusive, by making rust collections throughout Canada. Forms 2 and 5 have comprised 95 per cent. or more of the total isolations. For many years, White Tartar, a late maturing side oat. has been highly resistant to stem rust when grown in the spring wheat states. Six physiologic forms were isolated in Canada during the period from 1925 to 1927. While only a small percentage of the collections contains these virulent forms, there is the possibility of their gradual increase. The Minnesota station has produced a new oat variety named Anthony, which is highly re-

sistant to stem rust, a character obtained from its White Russian parent. It will be interesting to see if these virulent forms will spread and attack the new variety when it is introduced.

While physiologic forms make the breeding of disease-resistant varieties a more difficult problem, there is evidence available for the belief that the origin of new physiologic forms of pathogenic organisms obey known genetic laws. It has been demonstrated that these new forms may arise by either mutation or hybridization or both (Newton and Johnson, 1927; Craigie, 1927). If pathogenic fungi are as stable as their host plants, the contest of the future would seem to be waged on the basis of whether the breeder can develop desirable disease-resistant varieties of crop plants more rapidly than new physiologic forms of the organism causing the disease develop naturally. There is an apparent limit to the development of new physiologic forms based on the genotypic nature of forms now available. It would seem probable that most of the physiologic forms now present have been with us for some time, but that modern methods have been necessary to demonstrate them.

In the past, resistant varieties of crop plants have in many cases proved resistant for long periods of time. It appears probable that, in the future, greater progress can be made in breeding for disease resistance, by the isolation and use in breeding experiments of all available strains of the organism causing the disease, than was possible before the present-day view-point of the genetic nature of pathogenic organisms.

With this appreciation of the nature of pathogenic organisms, we may next turn our attention to the mode of inheritance in plants of reaction to particular diseases.

The Genetics of Disease Resistance in Plants

A summary of the large number of studies of the genetics of disease resistance would take a great deal of

time. An attempt will be made to present some of the different types of genetic behavior of reaction to diseases.

Two studies have been conducted in Minnesota to determine the mode of inheritance of resistance to stem rust in oats. In these studies, the White Russian type of resistance was used. One study consisted of crosses of White Russian with Minota or Victory, two mid-season open-panicled varieties (Garber, 1922). The other consisted of crosses between homozygous resistant selections from the first cross and Black Mesdag (Hayes *et al.*, 1928). In both crosses, resistance was dominant to susceptibility and segregation in F_2, and later generations proved that only a single factor pair for resistance and susceptibility was involved. There was no evidence of a close linkage of disease resistance with any other characters by which the parents differed. Recently, Dietz (1928) has studied a cross of White Russian, resistant to stem rust, with Burt, which was susceptible. In this cross, susceptibility was dominant, and in F_2 there was a ratio of 13 susceptible to 3 resistant. These results were explained on the basis of an inhibitory factor carried by the Burt parent which prevented the expression of the factor for resistance carried by the White Russian parent. These studies were conducted without paying particular attention to physiologic forms of rust.

Numerous studies of inheritance of reaction to stem rust of wheat have been made and, in many of these, individual physiologic forms of rust have been used. Kanred winter wheat is immune to eleven of the physiologic forms of rust which have been found in the United States. Immunity to these eleven forms is dominant over resistance, and segregation in F_2 occurs in the ratio of 3 immune to 1 susceptible. Immunity to all eleven forms of rust is dependent upon the same genetic factor (Aamodt, 1923).

Several crosses have been studied in Minnesota where the parents reacted in a reciprocal manner to two physio-

logic races of rust. These comprise crosses of durum × vulgare, durum × durum, and vulgare × vulgare. In each case, strains were isolated which were resistant to both rust forms as well as strains susceptible to both (Puttick, 1921; Harrington and Aamodt, 1923; Hayes and Aamodt, 1923).

In a study of reaction to rust form 1 to which Kanred is immune, Kota is resistant and Marquis is susceptible, Aamodt (1927) decided that the reaction in these three wheats to the same physiologic form was dependent upon a series of three multiple allelomorphs.

In studies of reaction of crosses between H–44–24 and Marquis, the first being moderately resistant to physiologic forms 9, 14, 17, 21 and 34 and moderately susceptible to form 15, and the latter resistant to form 14 but susceptible to the other forms, there was evidence that only a single factor pair was responsible for the reactions to all 6 forms (Goulden *et al.,* 1928). On this basis, it would be impossible to combine the resistance to form 14 of the Marquis parent with the moderate resistance of the H–44–24 parent to form 21.

In crosses between resistant wheats belonging to the 14 chromosome group of wheat species and susceptible common wheats of the 21 chromosome group, the 14 chromosomes of the Emmer group are homologues of 14 of the Vulgare group leaving 7 univalents. Sax recently (1928) stated that there was no evidence that it was possible to combine the desirable characters of vulgare and emmer wheats in a single variety. In earlier papers, he presented evidence (Sax, 1923) which led him to doubt the possibility that rust resistance could be transferred from varieties of *T. durum* to *T. vulgare* by crossing and subsequent selection. The first increase of Marquillo wheat for commercial distribution was made by the Minnesota station in 1927. Its rust resistance was obtained from Iumillo, a durum variety, its other parent being Marquis. In studies of resistance to stem rust where several

physiologic forms of rust were present in the nursery, there was evidence that resistance and susceptibility to all rust forms present were dependent upon two genetic factor pairs, 1 homozygous resistant line being obtained out of every 16 studied (Hayes *et al.*, 1925). These F_3 lines were grown from random selections of F_2 plants. In later generations, it was learned that some of the resistant lines were more resistant than Marquillo, while others were infected somewhat more severely than Marquillo. Besides the two pairs of factors, there was some evidence then that minor modifying factors were involved.

McFadden obtained highly resistant common wheats from crosses of Yaraslov Emmer and Marquis. One of these has been named Hope and another H44. In crosses, respectively, of Hope and H44 with Marquis, Clark *et al.* (1928) and Goulden *et al.* (1928) have obtained results indicating only a main pair of genetic factors involved in resistance and susceptibility, with resistance dominant. It appears probable that these Emmer selections carry a genetic factor for resistance to many and perhaps all forms of stem rust which were used in these studies. In crosses of H44 with Marquis, studied in Minnesota, there was some evidence that besides the main pair of factors minor modifying factors were involved, for lines were obtained which were somewhat less resistant than H44.

Gaines (1920, 1923, 1925), Gaines and Singleton (1926) and Briggs (1926) have studied the inheritance of resistance of wheats to bunt or covered smut caused by *Tilletia tritici*. The method used in studying bunt resistance was to blacken the seed with smut spores just before planting and study the individual crosses and strains in the segregating generations by the individual plant method. Some wheats such as Martin, Hussar and White Odessa are bunt-free and are considered immune. Turkey and Florence are highly resistant. Forty Fold and Red Russian are slightly resistant. Most bread wheats are susceptible. Crosses between susceptible

varieties have given only susceptible offspring, while crosses between resistant varieties have shown transgressive segregation, and from such crosses lines have been obtained which bred true for either immunity, resistance or susceptibility. Susceptibility is generally dominant to resistance, while crosses of the immune with the susceptible are resistant in F_1. Gaines has studied over twenty-five separate crosses and explains the results obtained in each on a multiple-factor basis. On the other hand, Briggs explained the results of one cross between immune and susceptible varieties by a single dominant factor for immunity.

When multiple factors are involved, it may be helpful to determine the genetics of resistance by studies of linkage relations with factors in known linkage groups. This method has been used in Minnesota for studies of the reaction to the "spot blotch" disease in barley caused by *Helminthosporium sativum.* In crosses of Lion and Manchuria, there was some indication of a linkage between resistance and susceptibility to the disease and other character pairs (Hayes *et al.,* 1923). Griffee (1925) studied this relation in a cross of Svanhals and Lion. The characters of the parents are as follows: Svanhals, 2-rowed, white glume, rough awn, resistant to *H. sativum;* Lion, 6-rowed, black glume, smooth awn, susceptible to *H. sativum.*

The character pairs, 2-rowed *vs.* 6-rowed, black *vs.* white and rough *vs.* smooth awns, were found to be independently inherited.

Resistance and susceptibility in F_2 tended to be correlated with each of these three character pairs. That the correlation is genetic may be inferred as breaks in the linkage relation have been found, and all combinations of resistance *vs.* susceptibility, black *vs.* white, 2-rowed *vs.* 6-rowed and smooth awns *vs.* rough awns have been obtained. This led to the conclusion that three genetic factor pairs or groups of factors are concerned with the manner of reaction to the "spot blotch" disease.

Similarly, it has been learned by Immer (1927) that resistance and susceptibility to attacks of *Ustilago zeae,* the corn smut organism, are dependent upon factors located in two of the eight linkage groups that have been differentiated.

Sufficient studies have been reviewed to prove that reactions to diseases are dependent upon similar modes of inheritance as other characters. In some cases, a simple Mendelian segregation is obtained. Resistance is dominant in some crosses, recessive in others. In other cases, many genetic factors condition manner of reaction.

THE NATURE OF RESISTANCE

Until recently, the geneticist has been satisfied with a knowledge of the mode of inheritance of the characters being studied. The physicochemical method by which a particular genetic factor causes the development of a certain character remains, for the most part, entirely unknown. In showing visitors resistant and susceptible strains of crop plants, one of the first questions which many people ask is, why is one variety resistant and another susceptible? What is the physiologic or morphologic cause?

It is evident that a knowledge of the cause would aid materially in attacking the problem of disease resistance, and some progress has been made in studying the causes of resistance. It has been shown for stem rust in wheat that there are two types of resistance. Stakman (1918) showed distinctly that resistance to physiologic forms of stem rust often is due to a real physiologic incompatibility. Even though the rust gained entrance into the host, it was unable to develop vigorously. Resistance of this nature is classified as a true protoplasmic resistance. Hursh (1924) decided that, in some cases, wheat varieties may be resistant to stem rust because of morphological peculiarities also. Rust mycelium develops almost exclusively in the collenchyma of wheat stems. In some varieties, the collenchyma bundles are small and sepa-

rated by sclerenchymatous fibers. Varieties with a large amount of sclerenchyma would be resistant. Hursh found that the resistance of Kota and Acme, for example, was a result of the large amount of sclerenchymatous tissue in their stems. While true protoplasmic resistance is perhaps more valuable than morphological resistance because it is less dependent upon environment, there is the possibility that morphological resistance would be less influenced by physiological forms of the disease organism than physiological resistance. It may be that the resistance of Marquillo and the segregates from McFadden's Emmer × Marquis hybrids are resistant to many and perhaps all forms of stem rust when the plants approach maturity because the resistance is morphological. This hypothesis is made solely on the basis of the resistance of these varieties to many physiologic forms when the plants approach maturity and because both Marquillo and Hope are susceptible to many forms of rust in the seedling stage.

The Wisconsin station has studied extensively the optimum temperatures for plant growth and the optimum temperatures for the development of the fungus (Jones *et al.*, 1926). It has been learned that soil temperatures greatly influence the severity of the attack of certain plant diseases. Using selfed lines of corn, Dickson and Holbert (1926) isolated lines, resistant and susceptible, to attacks of seedling blight caused by *Giberella saubinetti*. Corn seedlings, as a rule, remain healthy under conditions favorable to infection at temperatures of 24° C. Self-pollinated lines of corn were found to have very definite temperature limits within which they were capable of resisting seedling blights. Resistance and susceptibility appeared to be correlated with the ability to develop hexoses and polysaccharide building substances at various temperatures. Resistant strains could resist seedling blight at temperatures above 12° C.; normal corn has similar ability at temperatures above 24° C.,

while the seedlings of susceptible lines were susceptible at all temperatures. Aside from their general interest, these studies indicate that, by growing selfed lines under controlled temperature conditions and inoculating them with pathogenic organisms, resistant lines can be isolated.

These instances of the causes of resistance to diseases are sufficient to prove that information regarding the nature of resistance is important and may lead to better methods of isolating resistant varieties.

CONCLUSION

Plant diseases cause enormous losses, and some means of combating disease is often necessary. Disease resistance in plants is of great practical importance, and when resistance can be obtained the development and use of resistant varieties is the most satisfactory means of controlling diseases.

Stem rust of wheat and wilt in flax are diseases which, in the past, have limited the production of these crops. The development of stem rust resistant wheats and wilt resistant varieties of flax are illustrations of the possibilities of control of plant diseases by the development of disease-resistant varieties.

A knowledge of the genetic nature of disease organisms is as necessary as a knowledge of the genetic characters of the host plant. Many pathogenic organisms are composed of physiologic forms which can be differentiated in some cases only by their manner of reaction on particular host plants. These physiologic forms are dependent upon genotypic differences in the disease organisms. Physiologic forms do not change rapidly, and their genetic characters are as stable as those of higher plants. New physiologic forms may be produced by mutation or hybridization. The physiologic form concept has aided materially in placing the breeding of disease resistance on a sound basis.

Resistance and susceptibility are as stable as other genetic characters and are dependent upon genetic fac-

tors. In some cases, resistance and susceptibility are dependent upon a single genetic factor pair. In most studies of reaction to particular physiologic forms of stem rust of wheat where the parents reacted reciprocally to the forms, it was found possible to combine resistance to both forms in a single wheat strain. Resistance to stem rust in emmer and durum wheats has been transferred to vulgare wheats by crosses between varieties belonging to these two groups.

In many cases, resistance and susceptibility are dependent upon multiple factors. When resistance and susceptibility are dependent upon several genetic factors, an idea of the number of factors involved can be obtained by studies of linkage relations with factors in known linkage groups. By this method, it was learned that there were three factor pairs or groups of factors concerned with resistance *vs.* susceptibility to "spot blotch" in barley and two factor pairs or groups of factors for resistance *vs.* susceptibility to smut in maize.

A knowledge of the nature of resistance aids materially in a correct attack upon the problem of obtaining disease-resistant varieties. Instances of true protoplasmic resistance and of morphological resistance to stem rust of wheat have been found. Resistance to seedling blight in corn is dependent upon the metabolic response of the strains of corn under different temperatures.

Many questions remain to be solved. The progress made and the importance of the problem lead to the conclusion that the development of disease-resistant varieties is one of the most valuable methods of utilizing genetic principles in the solving of agricultural problems.

LITERATURE CITED

Aamodt, O. S.

1923. "The Inheritance of Growth Habit and Resistance to Stem Rust in a Cross between Two Varieties of Common Wheat," *Jour. Agr. Res.*, 24: 457–469.

1927. "A Study of Growth Habit and Rust Reaction in Crosses between Marquis, Kota and Kanred Wheats," *Phytopathology*, 17: 573–609.

Barker, H. D.
1923. "A Study of Wilt Resistance in Flax," *Minn. Agr. Exp. Sta. Tech Bull.* 20.

Briggs, F. N.
1926. "Inheritance of Resistance to Bunt, *Tilletia tritici* (Bjerk.) Winter, in Wheat," *Jour. Agr. Res.*, 32: 973–990.

Clark, J. A., and E. R. Ausemus
1928. "Immunity of Hope Wheat from Black Stem Rust Inherited as a Dominant Character," *Jour. Amer. Soc. Agron.*, 20: 152–159.

Craigie, J. H.
1927. "Experiments on Sex in Rust Fungi," *Nature*, 120: 116–117.

Dickson, James G., and James R. Holbert
1926. "The Influence of Temperature upon the Metabolism and Expression of Disease Resistance in Selfed Lines of Corn," *Jour. Amer. Soc. Agron.*, 18: 314–321.

Dietz, S. M.
1928. "Inheritance of Resistance in Oats to *Puccinia graminis avenae*," *Jour. Agr. Res.*, 37: 1–24.

Evans,
1911. "South African Cereal Rusts with Observations on the Problem of Breeding Rust-resistant Wheats," *Jour. Agr. Sci.*, 4: 95–104.

Gaines, E. F.
1920. "The Inheritance of Resistance to Bunt or Stinking Smut of Wheat," *Jour. Amer. Soc. Agron.*, 12: 124–132.
1923. "Genetics of Bunt Resistance in Wheat," *Jour. Agr. Res.*, 23: 445–480.
1925. "The Inheritance of Disease Resistance in Wheat and Oats," *Phytopathology*, 15: 341–349.

Gaines, E. F., and H. P. Singleton
1926. "Genetics of Marquis × Turkey Wheat in Respect to Bunt Resistance, Winter Habit and Awnlessness," *Jour. Agr. Res.*, 32: 165–181.

Garber, R. J.
1922. "Inheritance and Yield with Particular Reference to Rust Resistance and Panicle Type in Oats," *Minn. Agr. Exp. Sta. Tech. Bull.* 7.

Gordon, W. L., and D. L. Bailey
1928. "Physiologic Forms of Stem Rust in Canada," *Sci. Agr.*, 9: 30–38.

Goulden, C. H., and A. T. Elders
1926. "A Statistical Study of the Characters of Wheat Varieties Influencing Yield," *Sci. Agr.*, 6: 337–345.

Goulden, C. H., K. W. Neatby and J. W. Welsh
1928. "The Inheritance of Resistance to *Puccinia graminis tritici* in a Cross between Two Varieties of *Triticum vulgare*," *Phytopathology*, 18: 631–658.

Griffee, Fred.
1925. "Correlated Inheritance in Barley of Botanical Characters, and Manner of Reaction to *Helminthosporium sativum*," *Jour. Agr. Res.*, 30: 915–935.

Harrington, J. B., and O. S. Aamodt
1923. "Mode of Inheritance of Resistance to *Puccinia graminis* with Relation to Seed Color in Crosses between Varieties of Durum Wheat," *Jour. Agr. Res.*, 24: 979–996.

Hayes, H. K.
1923. "Inheritance of Kernel and Spike Characters in Crosses between Varieties of *Triticum vulgare*," *Univ. Minn. Studies Biol. Sci.*, No. 4: 163–183.

Hayes, H. K., and O. S. Aamodt
1923. "A Study of Rust Resistance in a Cross between Marquis and Kota Wheats," *Jour. Agr. Res.*, 24: 997–1012.

Hayes, H. K., O. S. Aamodt and F. J. Stevenson
1927. "Correlation between Yielding Ability, Reaction to Certain Diseases and Other Characters of Spring and Winter Wheats in Rod-row Trials," *Jour. Am. Soc. Agron.*, 19: 896–910.

Hayes, H. K., F. Griffee, F. J. Stevenson and A. P. Lunden
1928. "Correlated Studies in Oats of the Inheritance of Reaction to Stem Rust and Smuts and of Other Differential Characters," *Jour. Agr. Res.*, 36: 437–457.

Hayes, H. K., J. H. Parker and Carl Kurtzweil
1920. "Genetics of Rust Resistance in Crosses of Varieties of *Triticum vulgare* with Varieties of *T. durum* and *T. dicoccum*," *Jour. Agr. Res.*, 19: 523–542.

Hayes, H. K., and E. C. Stakman
1922. "Wheat Stem Rust from the Standpoint of Plant Breeding," *Proc.* Second Annual Meeting Canadian Soc. Agron., 1922: 22–35.

Hayes, H. K., E. C. Stakman and O. S. Aamodt
1925. "Inheritance in Wheat of Resistance to Black Stem Rust," *Phytopathology*, 15: 371–387.

Hayes, H. K., E. C. Stakman, Fred Griffee and J. J. Christensen
1923. "Reaction of Barley Varieties to *H. sativum*," *Minn. Agr. Exp. Sta. Tech. Bul.* 21.

Hursh, C. R.
1924. "Morphological and Physiological Studies on the Resistance of Wheat to *Puccinia graminis tritici* Erikss. and Henn., *Jour. Agr. Res.*, 27: 381–411.

Immer, F. R.
1927. "The Inheritance of Reaction to *Ustilago zeae* in Maize," *Minn. Technical Bull.* 51.

Immer, F. R., and F. J. Stevenson
1928. "A Biometrical Study of Factors Affecting Yield in Oats," *Jour. Am. Soc. Agron.*, 20: 1108–1119.

Jones, L. R., and J. C. Gilman.
1915. "The Control of Cabbage Yellows through Disease Resistance," *Wis. Agr. Exp. Sta. Res. Bul.*, 38.

Jones, L. R., James Johnson and J. G. Dickson.
1926. "Wisconsin Studies upon the Relation of Soil Temperature to Plant Disease," *Wis. Res. Bul.* 71, 144 pp.

Jones, L. R., J. C. Walker and W. B. Tisdale.
1920. "Fusarium-resistant Cabbage," *Wis. Agr. Exp. Sta. Res. Bul.*, 48.

Kostoff, Dontcho.
1928. "Induced Immunity in Plants," *Nat. Acad. of Sciences*, 14: 236–237.

Newton, Margaret, and T. Johnson.
1927. "Color Mutations in *Puccinia graminis tritici* (Pers.) Ericks. and Henn.," *Phytopathology*, 17: 711–725.

Puttick, G. F.
1921. "The Reaction of the F_2 Generation of a Cross between a Common and a Durum Wheat to Two Biologic Forms of *Puccinia graminis*," *Phytopathology*, 11: 205–213.

Sax, Karl.
1923. "The Relation between Chromosome Number, Morphological Characters and Rust Resistance in Segregates of Partially Sterile Wheat Hybrids," *Genetics*, 8: 301–321.

Sax, Karl.
1928. "Chromosome Behavior in Triticum Hybrids," *Zeits. ind. Abs. und Vererb.*, 11: 1267–1284.

Stakman, E. C.
1926–27. "Racial Specialization in Plant Disease Fungi," "Plant Pathology and Physiology in Relation to Man," pp. 92–150. Saunders and Co., Philadelphia and London.

Stakman, E. C., J. H. Parker and F. J. Piemeisel.
1918. "Can Biologic Forms of Stem Rust on Wheat Change Rapidly Enough to Interfere with Breeding for Rust Resistance?" *Jour. Agr. Res.*, 14: 111–123.

Stakman, E. C., F. J. Piemeisel and M. N. Levine.
1918. "Plasticity of Biologic Forms of *Puccinia graminis*," *Jour. Agr. Res.*, 15: 221–249.

Waldron, L. R.
1926. "Hybrid Selection of Marquis and Kota," *N. Dak. Agr. Exp. Sta. Bul.* 200.

EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

XIII. THE INFLUENCE OF DIFFERENT FEEDING DURING THE LARVAL AND IMAGINAL STAGES ON THE DURATION OF LIFE OF THE IMAGO OF *DROSOPHILA MELANOGASTER*[1]

W. W. ALPATOV[2]

I

ALTHOUGH the fruit-fly has been for almost thirty years a favorite object of experimental entomology (see Castle, Clark, Mast and Barrows, 111), the first accurate paper on duration of life of this insect was published only in 1921 (Pearl and Parker, 21), opening a new period in the history of the experimental study of duration of life in general and that of insects in particular. Sufficient time has passed since that publication to justify the making of a short review of all attempts in this field. This is done in summarized form in Table I.

It can be seen from this table how diversified are the factors which have been studied in their application to the duration of life. At the same time it is evident that all these efforts are far from being sufficient to give us a complete picture of the relationship of different factors to the duration of life. For instance, a whole group of external factors like radiant energy of different wave-length, electricity and magnetism has never (with one exception—Northrop) been studied in relation to longevity. The feeding, and particularly the influence of different foods on duration of life, has also never been tested on *Drosophila*. Other insects like bees (Phillips, 118) proved to be excellent material for studies of influence of different foods on duration of life. Another point to be emphasized is that even in the cases of thor-

[1] From the Institute for Biological Research of the Johns Hopkins University.

[2] Research fellow of the International Education Board.

TABLE I

REVIEW OF THE QUANTITATIVE DATA RELATING TO THE INFLUENCE OF DIFFERENT FACTORS UPON THE DURATION OF LIFE IN *Drosophila melanogaster*

Groups of factors	Factor	Author	Year	Influence found or not and its character
Genetic factors	Mutation	Hyde	1913	Shortens the duration of life.
	Line breed strains	Pearl and Parker	1922	Short and long lived lines.
	Mutations	Pearl, Parker and Gonzalez	1923	Shorten the duration of life.
	Mutations	Gonzalez	1923	Shorten the duration of life.
Temperature	Temperature of imaginal life	Loeb and Northrop	1917	Low temperature increases the duration of life.
	Temperature of imaginal life	Alpatov and Pearl	1929	Low temperature increases the duration of life.
	Temperature of the development	Alpatov and Pearl	1929	Low temperature increases the duration of life.
Humidity	Ventilation	Pearl and Parker	1922	Live longer in ventilated vials.
	Absorbent paper in food	Pearl and Parker	1921	No effect.
Light	.....	Northrop	1925	Above 100 meter candles the duration of life is rapidly shortened.
Clipping the wings	.....	Pearl, Miner and Parker	1927	No effect.
Narcotics	Etherization	Pearl and Parker	1922	No effect.
Food and feeding	Starvation and 5 per cent. alcohol	Sekla	1928	Alcohol prolongs the duration of life.
	Starvation with water	Lutz	1915	Shortens the life.
	Complete starvation	Pearl and Parker	1924	Shortens the life.
	Intermittent starvation	Kopeč	1928	Shortens and (?) prolongs the duration of life.
	Prolongation of larval life by underfeeding	Northrop	1917	No effect on imaginal life.
	Embryonic juice and larval pulp	Pearl and Parker	1922	No effect.
	Agar + water, salt and dextrol or "glucose-agar"	Loeb and Northrop	1917	Life longest on "glucose-agar."
Aseptic culture	Aseptic larval and pupal life	Steinfeld	1928	Aseptic conditions prolong the duration of life.
	Aseptic whole life			
Density of population	Density of population	Pearl and Parker	1922	
		Pearl and Parker	1923	There is an optimal density of population.
		Pearl and Parker	1927	
Duration of embryonic period (egg-larval period)	.....	Lutz	1915	Negatively related to the duration of life.

ough study of the influence of a particular factor on the duration of life the character of the functional relationship between the particular factor and the duration of life has usually remained undiscovered. Exceptions are such factors as temperature (Loeb and Northrop; Alpatov and Pearl), density of population (Pearl, Miner and Parker, 117) and light (Northrop, 115). Unfortunately, the last paper gives only a very abbreviated summary of the results, without showing the original data or even the calculated constants.

These remarks give a certain justification for showing in Fig. 1 curves based on data published by Kopeč (114).

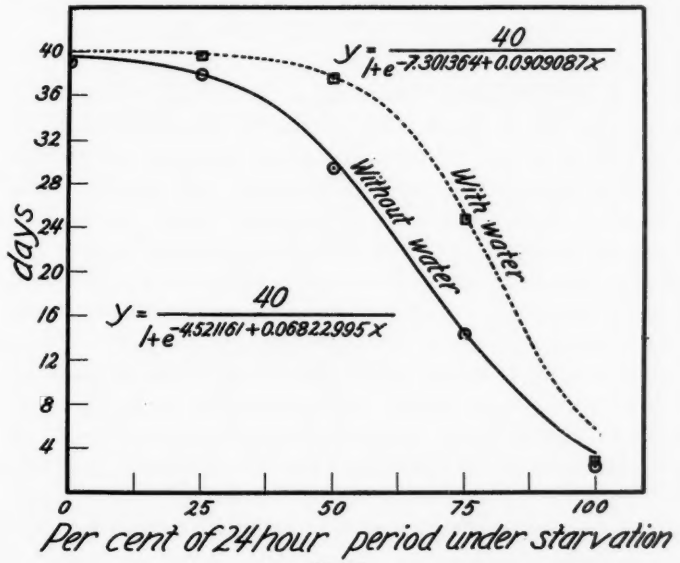

FIG. 1

The upper curve represents the relation between the duration of life at different grades of intermittent starvation with water, and the lower one represents the same but for experiments when water was not supplied. The period of transferring the flies from food in empty bottles was equal to twenty-four hours—flies being kept without food six, twelve and eighteen hours—which we

expressed as 25 per cent., 50 per cent. and 75 per cent. starvation. The curves, which fit very well, particularly the left portion of the observed points, belong to the type of logistic curves.[3] As far as the right-hand portion of the observed data is concerned (near the very high degree of starvation) there is not sufficient evidence to be sure that there is here a trend corresponding to the branch of logistic curve with a decreasing slope. But on the whole these curves differ entirely from the exponential curve which represents the relation of duration of life to temperature (Alpatov and Pearl, 110) as well as that of duration of life and density of population (Pearl, Miner and Parker, 117).

II

Our present investigation is to be considered as a preliminary study for a future detailed investigation of the influence of nutrition on the duration of life of *Drosophila*. Our material is based on three independent duration of life experiments. The first of these consisted in producing small size flies by taking the larvae from the food before the normal end of larval feeding and testing their longevity. The two others give information concerning the duration of life of flies kept on synthetic food without yeast, and the influence of changing food every day as compared with every second day.

The preparation of the material for the first experiment consisted in putting 0–4-hour-old larvae collected according to a method previously described (108) in half-pint bottles containing 100 cc synthetic medium, with yeast planted the day before. One hundred larvae of wild flies line 107 were put in each bottle. The larvae were kept at a temperature of 25° C. Thirteen bottles with 100 larvae in each bottle produced 1,033 adult flies—535 females and 498 males. That means that 79.5 per cent. of the larvae reached the adult stage, the number

[3] The method used to fit the logistic curves is described by Reed and Berkson (p. 767, 119).

of males being equal to 93.1 of the females. The control flies emerging from normally fed larvae were placed in one-ounce duration of life bottles, fifteen males and fifteen females in each, except one which had ten males and twenty females. The total number of flies for which death has been recorded may be found in Table III.

The underfed larvae were kept in the same way as the normally fed until they had fed fifty-nine hours. At that time they were taken from the food and placed in bottles with plain 2 per cent. agar-agar washed in distilled water. The mouths of the bottles were covered with 40 mm watch glasses sealed with plasteline until the moment of pupation when the watch glasses were again replaced by the usual cotton stopper. This was done to prevent the larvae from crawling out as they are apt to do before pupation. Out of 1,745 larvae put on plain agar-agar 1,243 adult flies emerged—651 females and 592 males—which shows that out of fifty-nine-hour-old larvae only 71.2 per cent. reached the final stage. In other words, there is a comparatively high mortality among the larvae unable to pupate. The males were equal in number to 90.9 per cent. of the females. The same density in duration of life bottles as in the controls was used for underfed flies, except in one bottle where thirteen males and seventeen females were kept together, and two others with thirty females each. The density in the rest of the bottles was fifteen males and fifteen females per bottle. The experience of this institute does not give any conclusive indications about the influence of celibacy on duration of life. Therefore we assume the right to include in our life table calculations the records of these sixty females kept without males. Records of the number of dead males in five bottles and of females in three bottles were larger than the original number of flies put in the bottles in the beginning of the experiments and were therefore discarded from the calculations. Food was changed every day except Sundays. We will not go into the history of the question of influence of underfeeding

of larvae upon the size of the imago, another paper (109) being partly devoted to this question.

III

Table II represents the basic biometrical constants of the measurements of the wing length and width of our underfed and normally fed flies. The measurements were made according to the scheme described before (110). Two most important facts have to be emphasized. First, that the reduction in the size of the wing is much more pronounced in females than in males. It can be judged from the values of the ratios and also from the expression of the length of wings of underfed flies in proportion to the length of wings of normally fed ones. This expression for females is equal to 83.3 and for males it is equal to 92.0. This peculiarity has its explanation in the fact that the difference in size between larvae which will produce males and females becomes more and more pronounced as the larvae approach the pupal stage. In other words, the larvae which will produce males are closer to the final larval size at a given moment of the larval life than the larvae which will become female imagoes. Therefore, younger larvae taken from the food produce males which are closer to normal males than the corresponding females to normally fed females. The second striking difference is the much larger variability of the underfed flies. This greater variation of experimental flies is in accord with a long-known fact that abnormal, unfavorable conditions increase the variation (see Pearl, 116). It is interesting to note that in case of wing width the underfed males have even broader wings than the corresponding females. The sex difference between the underfed flies is almost negligible as compared with that in normally fed flies. Tables III and IV and Figs. 2 and 3 represent the results obtained. The underfed females show the same duration of life as the control ones. The males show a longer duration, although the difference is statistically not very signifi-

TABLE II

BIOMETRIC CONSTANTS OF FLIES DEVELOPED FROM UNDERFED AND NORMALLY FED LARVAE IN MILLIMETERS

		Females from normally fed larvae	Difference and Ratio $\frac{\text{Diff.}}{\text{P.E.}}$	Females from underfed larvae	Males from normally fed larvae	Difference and Ratio $\frac{\text{Diff.}}{\text{P.E.}}$	Males from underfed larvae
Length of the wing	Mean	1.738 ± .005	.291 ± .013 R = 22.4	1.447 ± .012	1.532 ± .004	.123 ± .008 R = 15.4	1.409 ± .007
	Standard deviation	.0398	—	.1246	.0358	—	.0770
	Coefficient of variation	2.29 ± .21	—	8.61 ± .60	2.34 ± .18	—	5.46 ± .37
	N	25	—	47	40	—	50
Width of the wing	Mean	.9969 ± .0030	.1844 ± .0076 R = 24.3	.8125 ± .0070	.8962 ± .0029	.0800 ± .0052 R = 15.4	.8162 ± .0043
	Standard deviation	.0223	—	.0709	.0270	—	.0447
	Coefficient of variation	2.24 ± .21	—	8.73 ± .61	3.01 ± .23	—	5.48 ± .37
	N	25	—	47	40	—	50

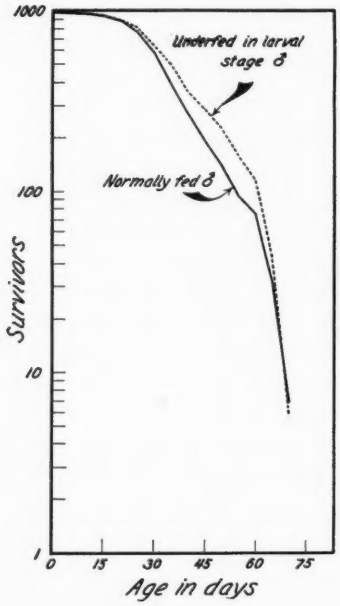

FIG. 2

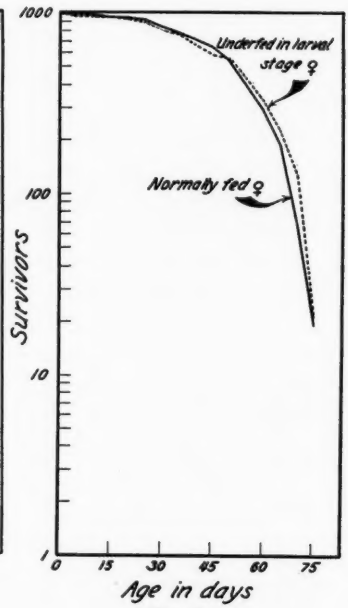

FIG. 3

cant. The conclusion which may be drawn is that in spite of the very significant reduction in size (for the female wing length 16.7 per cent., wing width 18.5; for the male wing length 8.0, wing width 8.9) the duration of life remains about the same. Comparing these results with the conclusions drawn in a paper on temperature (110) where it was shown that cold flies, characterized by larger body size, have at the same time a greater longevity than the control ones (in this case the reduction in size for four characters was for males equal to 10.1 per cent., and to 7.8 for females) it must be admitted that the size of body itself is not the fundamental factor determining the duration of life. The same somatological effect—reduction of body size—when produced by different factors (in our first case by temperature, in the present case by undernourishment) gives quite different results.

TABLE III

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES UNDERFED AND NORMALLY FED IN THE LARVAL STAGE

Days	Underfed flies		Normally fed flies	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	987	976	989	989
10–14	979	967	975	981
15–19	949	950	945	965
20–24	887	935	897	960
25–29	782	886	814	928
30–34	646	803	593	846
35–39	500	762	393	785
40–44	361	677	271	718
45–49	288	585	192	651
50–54	223	523	140	534
55–59	158	426	95	398
60–64	115	327	75	290
65–69	42	220	32	187
70–74	6	128	7	69
75–79	0	19	0	21
80–84	—	0	—	0
Mean	37.11 ± .52	48.42 ± .54	34.59 ± .44	49.11 ± .50
Standard deviation	15.26	18.41	13.59	16.12
Coefficient of variation	41.12 ± 1.14	38.02 ± .89	39.29 ± 1.02	32.82 ± .79
Absolute number of flies	397	532	444	474

We want to emphasize the danger of fallacious conclusions. It is not entirely improbable that a little greater duration of underfed males and an equal duration of underfed females originated as a result of a certain selective process. Only the strongest larvae succeed in the struggle for life without a sufficient supply of food. Table IV contains also some indices calculated by the well-known approximate formula recommended by Johannsen (113, p. 706). There is a slight reduction of the sex differences in duration of life among underfed flies as compared with normal ones.

TABLE IV

AVERAGES AND INDICES OF THE DURATION OF LIFE OF FLIES UNDERFED IN LARVAL STAGE AND NORMALLY FED

	Underfed in larval stage	Difference and Ratio	Normally fed in larval stage
	Average duration of life		Average duration of life
Female ..	48.42 ± .54	.69 ± .74 R = .93	49.11 ± .50
Male	37.11 ± .52	2.52 ± .68 R = 3.7	34.59 ± .44
	Indices		Indices
Male	76.65 ± 1.37	6.22 ± 1.79 R = 3.5	70.43 ± 1.15
Female ..	100	—	100
Male	107.30 ± 2.02	8.71 ± 2.51	100
Female ..	98.59 ± 1.49	R = 3.5	100

IV

The next experiment originated from a purely practical question. In our experiments on egg production of the fruit-fly we used the synthetic food as a substratum given to females on which to deposit eggs. To strew yeast on the surface was a great nuisance because it hampered us in counting the eggs. We finally decided to put some drops of yeast suspension on the surface of the synthetic food. But the question whether the presence of yeast influences the duration of life of adult flies yet remained unsolved. The question is, in other words, whether the carbohydrates available in synthetic food (the new synthetic medium contains 8.3 per cent. of cane sugar) are sufficient to keep up the life of an adult *Drosophila.*

We must admit that the experiment was not perfect from one point of view. The flies used came from ordinary room temperature mass culture, while undoubtedly it would be much more desirable to work with flies developed under complete absence of any micro-organisms. In the beginning of the experiment the surface of the food in bottles without yeast remained during twenty-

four hours just as shiny as at the moment of putting the flies in the bottles. Toward the middle of the experiment there could be observed in bottles a certain kind of micro-organism growth. But this slight growth was entirely different from the usual growth of yeast on the surface of the synthetic medium.

The flies used in this experiment were taken from wild line 107 on the fifth and sixth days after the beginning of the emergence in the corresponding bottles. Naturally their age at the moment of the beginning of the experiment was equal to 0–24 hours. The food in experimental and control sets of bottles was changed every day including holidays. The density was twenty-five males and twenty-five females per bottle. In the series kept without yeast we included also a bottle with thirty-two females kept without males, and a bottle with fifty females which were put without males according to the record taken at the moment of starting the experiment,

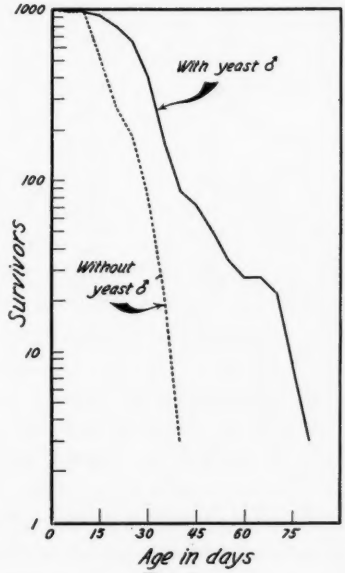

FIG. 4

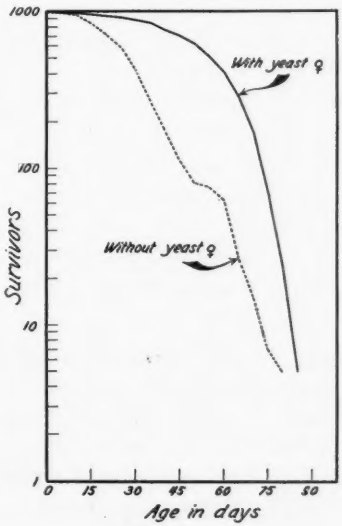

FIG. 5

but according to the subsequent observations on the process of dying out it turned out that the bottle contained also eight males. The series with yeast, besides normally populated bottles (twenty-eight males and twenty-five females), includes one which had thirty-two females kept separately, and another which had twenty females and twenty males.

The results are brought together in Tables V and VI and represented graphically on Figs. 4 and 5. It can be seen at once that the flies kept without yeast live a much

TABLE V

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES KEPT ON SYNTHETIC FOOD WITH AND WITHOUT YEAST

Days	Food without yeast		Food with yeast	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	983	986	978	985
10–14	969	955	973	980
15–19	523	842	924	960
20–24	276	722	808	948
25–29	186	590	655	933
30–34	82	420	400	895
35–39	23	271	171	845
40–44	3	175	88	772
45–49	0	113	73	717
50–54	—	82	51	625
55–59	—	77	35	517
60–64	—	63	27	412
65–69	—	27	27	289
70–74	—	15	22	171
75–79	—	7	8	71
80–84	—	5	3	23
85–89	—	0	0	5
90–94	—	—	—	0
Mean	17.725 ± .258	29.25 ± .48	28.715 ± .417	53.24 ± 1.80
Standard deviation	7.220	14.625	11.92	17.58
Coefficient of variation	40.73 ± 1.03	50.00 ± 1.43	41.51 ± 1.19	33.02 ± .87
Absolute number of flies	356	417	372	399

TABLE VI

AVERAGES AND SEX INDICES OF THE DURATION OF LIFE OF FLIES KEPT WITHOUT YEAST AND WITH YEAST

	Without yeast Average duration of life	Difference and ratio	With yeast Average duration of life
Females	29.25 ± 0.48	23.99 ± 1.90 R = 12.63	53.24 ± 1.80
Males	17.725 ± 0.258	10.99 ± 0.490 R = 22.43	28.715 ± 0.417
	Indices		Indices
Males	60.60 ± 1.33	6.66 ± 2.39 R = 2.79	53.94 ± 1.98
Females	100		100
Males	61.73 ± 1.26	6.79 ± 2.41	100
Females	54.94 ± 2.06	R = 2.81	100

shorter time than the control ones. If we compare the sex index of duration of life (meaning the male duration of life expressed in per cent. of the female) of our second experiment with those of the first (underfed and normally fed flies) we observe that in the former the index is significantly higher than in the latter. We have no data to explain this difference, leaving it for further investigation.

Up to the present time most of the authors working on nutrition of flies have concentrated their whole attention on the requirements of larvae for different nutritive substances. Little attention has been paid to the adult form. There are meager data published by Guyénot (112), Loeb and Northrop (16), Vinokuroff (70) and Glaser (75, 76). The first of these authors was mainly interested in the influence of different kinds of food on reproduction. Loeb and Northrop could not show any difference between the duration of life of flies kept on glucose-agar with yeast and without it. Vinokuroff's data show that the average duration of life of flies kept on sugar with addition of peptone is higher than without it (22 days against 17.6). Glaser's conclusions based on a very small number of experimental animals are not very definite.

His statement is: "On a diet of sucrose and bouillon, sucrose and blood serum, glucose and bouillon, glucose and blood serum, the longevity and degree of egg deposition reach their maximum." This means that carbohydrates alone are not sufficient for adult insects which live longer on food with the addition of proteins. In our case very likely such proteins have been supplied by the growing yeast cells. We are perfectly well aware of the fact that a whole series of experiments would be needed to clear up entirely the question whether the proteins could be given in another form than living yeast cells.

V

The last experiment with different feeding arose as a side issue of an attempt to determine the influence of light and darkness, as well as of intermittent light, on the duration of life of *Drosophila*. All three groups were

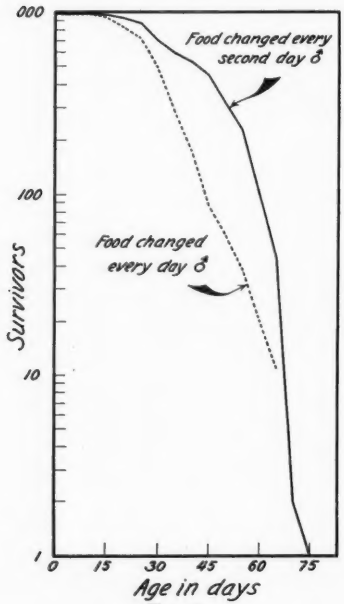

FIG. 6

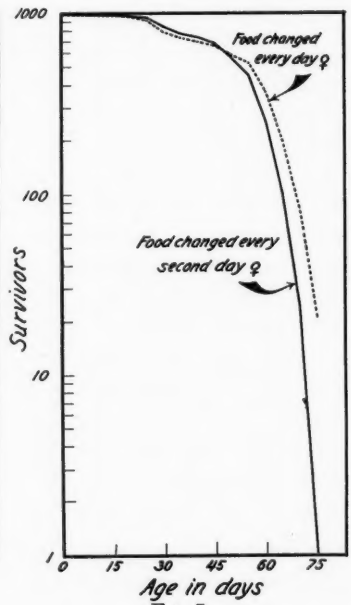

FIG. 7

kept in one incubator and in each of these groups half of the bottles were changed every day (except Sunday) and the other half three times a week. The differences in duration of life under different conditions of illumination were found to be statistically insignificant, which permitted the combining of all flies with similar conditions of food changing, and the comparison of their duration of life with each other. The density in this experiment was twenty-five males and twenty-five females per bottle. Tables VII and VIII and Figs. 6 and 7 show that there is no influence on female duration of life in experimental and control groups. On the other hand, the males in the

TABLE VII

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES KEPT ON FOOD CHANGED EVERY DAY AND EVERY SECOND DAY

Days	Food changed every day		Food changed every second day	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	996	997	999	998
10–14	992	992	992	993
15–19	950	981	970	984
20–24	836	967	938	978
25–29	722	936	878	961
30–34	491	802	701	848
35–39	280	750	601	791
40–44	173	715	538	756
45–49	86	677	447	686
50–54	59	598	329	571
55–59	38	538	228	468
60–64	20	378	92	251
65–69	12	200	43	101
70–74	0	84	2	24
75–79	.....	21	1	1
80–84	.....	0	0	0
Mean	30.775±0.274	50.68±0.42	41.295±0.354	49.555±0.341
Standard deviation	11.03	16.77	14.66	14.22
Coefficient of variation	35.84 ±0.71	33.09±0.64	35.50 ±0.68	28.70 ±0.52
Absolute number of flies	735	732	780	793

TABLE VIII

AVERAGES AND INDICES OF THE DURATION OF LIFE OF FLIES KEPT IN BOTTLES WITH FOOD CHANGED EVERY DAY AND EVERY SECOND DAY

	Every day change Average duration of life		Every second day change Average duration of life
Females ...	50.68 ± .42	1.125 ± 0.54 R = 2.1	49.555 ± .341
Males	30.775 ± .274	10.520 ± .448 R = 23.5	41.295 ± .354
Males	60.72 ± .74	22.6 ± 1.18 R = 19.2	83.33 ± .92
Females ...	100	.....	100
Males	74.52 ± .92	27.75 ± 1.43	100
Females ...	102.27 ± 1.10	R = 19.4	100

group where the food was changed every day show a very low longevity as compared with the controls. There is no doubt about the statistical significance of this difference in this particular experiment. It is remarkable that the duration of life sex index is extremely high for the males in the control group. Even if we compare the male mortality in the group in which the food was changed every day with the control group of our first experiment, and with flies normally fed in the larval stage, the difference remains significant, showing exceptionally low duration of life of males in case the food is changed every day.

Discussing the previous experiment we tried to point out that yeast plays an important rôle in prolongation of the life of *Drosophila melanogaster*. Very likely it is not the dry yeast cells which are eaten by the flies but fresh yeast growth which appears in abundance particularly twenty-four hours after the preparation of the food. Changing the food every day we evidently kept our experimental flies on a kind of intermittent partial starvation, depriving them of luxuriantly grown yeast colonies. This is one of the most plausible explanations of the shorter duration of males on food changed every day.

Why the female did not show the same effect can be perhaps answered by taking into account the differences in female constitution and physiology. The fat body, which represents the place of storing the nutritive substances in insect organisms, is much better developed in females than in males. Besides that, the egg-producing activity in females must react quite differently to the same external factor which first seemed to be responsible for the reduction of male life in case of every day change. A question may arise whether the effect is due to the influence of a purely mechanical shaking, which takes place much more often in the group changed every day as compared with the controls. This difference must be particularly pronounced in the beginning of the experiment. Afterwards the taking out of dead flies even without changing food requires shaking them out and back. We do not think this factor is to be taken into very serious consideration because the handling of the flies has been done by ourselves (W. W. A.) with extreme care and attention. On the other hand, against the possibility of such an explanation is the fact that the whole span of life of control flies is much longer than that of the experimental flies, showing that the process of dying was going differently all the time even when the difference in frequency of shaking the bottles belonging to the two groups disappeared almost entirely.

Summary

Summarizing, it has been shown in this paper that:

(1) The relation between the duration of life and different factors is expressed by quite different types of curves. Temperature and duration of life are connected by a simple exponential curve, while starvation (data from Kopeč) and duration of life at different grades of intermittent starvation can be represented by the upper part of a logistic curve.

(2) *Drosophila* females emerged from larvae taken from the food before the end of the normal larval feed-

ing do not differ in their duration of life from the controls. The males show even a longer (although statistically not very significant) duration of life as compared with the controls. This shows that a reduction in body size does not lead inevitably to a reduction of the duration of life, as has been the case with "room" and "cold" temperature flies which differed in size of the body and of the duration of life.

(3) Keeping flies on synthetic food with yeast and without it indicates that absence of yeast reduces greatly the duration of life of males and females. Very likely the carbohydrates available in the synthetic food are not sufficient for nutrition of adult *Drosophila,* and additional substances included in living yeast cells are required.

(4) Changing synthetic food with yeast every day and every second day indicates that the female duration of life is not affected by this procedure, while males in this experiment show a much shorter duration of life when transferred to new bottles every day. This difference may perhaps be attributed to sex differences in metabolism, or food requirements of male and female organism. Every day food changing can possibly be considered as a partial starvation, because yeast shows growth only after twenty-four hours, and changing food every day does not permit the flies to have yeast growth in such abundance as in the case when the bottles are changed every second day. It is also possible that the results for the males in this particular experiment are not typical.

LITERATURE CITED

(The plan of numbering citations is explained in the second of these studies, AMERICAN NATURALIST, 56: 174.)

108. Alpatov, W. W., "Growth and Variation of the Larvae of *Drosophila melanogaster*," *Jour. of Exp. Zool.*, Vol. 52, No. 3, pp. 407–432, 1929.

109. *Id.* "Phenotypical Variation in Body and Cell Size of *Drosophila melanogaster*" (in press).

110. Alpatov, W. W., and Pearl, Raymond, "Experimental Studies on the Duration of Life. XII. Influence of Temperature during the Larval Period and Adult Life on the Duration of the Life of the Imago of *Drosophila melanogaster*," AMERICAN NATURALIST, 63: 37–67, 1929.

111. Castle, W. E., Carpenter, F. W., Clark, A. H., Mast, S. O., and Barrows, W. M., "The Effect of Inbreeding, Cross-breeding and Selection upon the Fertility and Variability of *Drosophila*," *Proc.* Amer. Acad. of Arts and Sciences, Vol. XLI, No. 33, pp. 731–786, 1906.

112. Guyénot, L., "Recherches sur la vie aseptique et le développement d'un organisme en fonction du milieu," Thesis, Paris, 330 pp. + 4 plates, 1917.

113. Johannsen, W., "Elemente der Exakten Erblichkeitslehre," third edition, 1926.

114. Kopeč, Stefan, "On the Influence of Intermittent Starvation on the Longevity of the Imaginal Stage of *Drosophila melanogaster*," *British Jour. Exp. Biology*, Vol. V, No. 3, pp. 204–211, 1928.

115. Northrop, J., "The Influence of the Intensity of Light on the Rate of Growth and Duration of Life in *Drosophila*," *Jour. of Gen. Phys.*, Vol. IX, No. 1, pp. 81–86, 1925.

116. Pearl, R., "Variation in *Chilomonas* under Favorable and Unfavorable Conditions," *Biometrika*, 5: 53–72, 1906.

117. Pearl, R., Miner, J. R., and Parker, S. L., "Experimental Studies on Duration of Life. XI. Density of Population and Life Duration in *Drosophila*," AMERICAN NATURALIST, 61: 289–318, 1927.

118. Phillips, E. F., "The Utilization of Carbohydrates by Honey-bees," *Jour. of Agric. Research*, Vol. 35, No. 5, pp. 385–428, 1927.

119. Reed, L., and Berkson, J., "The Application of the Logistic Function to Experimental Data," *Jour. of Physical Chemistry*, 33: 760–779, 1929.

120. Sekla, B., "Experiments on Duration of Life in *Drosophila*" (in Czech, with English summary), *Časopic lék. čes*, 67: 85, 1928.

THE UTILIZATION OF AQUATIC PLANTS AS AIDS IN MOSQUITO CONTROL

PROFESSOR ROBERT MATHESON

CORNELL UNIVERSITY

OUR knowledge of mosquitoes has been acquired in very recent years. Previous to 1898, the year in which Sir Ronald Ross announced his epochal discovery that "dappled-winged" mosquitoes are the intermediate hosts of the malarial parasites, scarcely anything was known of these fiercely biting foes of man and animals. Their blood-thirstiness was well known, for in many portions of the earth man was unable to withstand their attacks and some of our fairest regions had to be abandoned to these tiny victors. Even to-day man is rather helpless before them. The writings of explorers, travelers, etc., often contain accounts that give us a picture of the fierceness and terribleness of a mass mosquito attack. Since 1898 we have acquired an immense amount of detailed information about the disease relationships, habits, life histories, biology, etc., of mosquitoes. To indicate briefly some of these advances seems worth while before attempting to outline the importance that plants may play in solving the problem of mosquito control.

Previous to 1896 the life history of practically only a single species, the common house mosquito (*Culex pipiens*), was known. In 1900 Dr. L. O. Howard published the first account of the life history of an American anopheline. As *Anopheles* species are the vectors, and the only known vectors, of malaria, the study of these mosquitoes has been very intensive, especially since the world war. Malaria is probably one of the most widespread and most serious of all human diseases. Though quinine and its various derivatives are used extensively in alleviating the attacks of malaria they are not specific cures, and the only effective method is the control of the

anopheline carriers. In 1900 Dr. Walter C. Reed and his associates announced the discovery that yellow fever is conveyed from man to man by a mosquito, the so-called tiger mosquito (*Aedes aegypti* or *Aedes argenteus* or *Stegomyia fasciata*). This discovery greatly increased the activities of the students of mosquito biology. Yellow fever is endemic to the Americas but was established in west Africa sometime before 1900. Recent outbreaks in Africa have aroused world-wide interest. Until 1928 it was confidently stated that only the tiger mosquito was the vector of yellow fever. Bauer (1928) has demonstrated that at least three other species may be vectors in west Africa. Long before this (1879) Sir Patrick Manson had shown that a round worm (*Filaria bancrofti*) of man had a mosquito as its host for part of its life-cycle. It was not, however, till 1900 that the method of transfer of this parasite was finally established. The variety of diseased conditions produced in man by this parasite is even yet not well known. This parasite is wide-spread throughout the tropical and subtropical regions of the world.

Dengue, a febrile disease of man which often appears in epidemic form in the tropical and subtropical regions, was shown in 1903–04 to be transmitted by a mosquito (*Culex fatigans*). Recent work seems to prove conclusively that *Aedes argenteus,* the yellow fever mosquito, is probably the only vector. In addition to these diseases of man, several animal diseases are transmitted by mosquitoes.

The knowledge that mosquitoes are the vectors, and probably the only vectors, of these serious diseases of man has led to a world-wide intensive study of mosquito biology. In 1900 some 242 species were known from the world. To-day probably over 3,000 species are recorded. All known species of mosquitoes pass their larval stages in water. They are known to breed in a great variety of aquatic environments—in ponds, slow-flowing streams, swamps, salt marshes, pools formed by melting snows,

all sorts of artificial water containers, foul and clear water, reservoirs, rice fields, irrigation ditches, water in tree holes, in the water contained by the leaves of epiphytic bromeliads, in the water in the leaves of pitcher plants, etc., etc. However, from all these studies we are beginning to learn what are the aquatic conditions essential for the breeding of each species of mosquito. This has led to more intensive studies of certain species to determine why certain types of aquatic environment are selected, and others, apparently identical as far as we can judge, are avoided. It was early recognized that not all types of water were selected by mosquitoes, but it was not till the past few years that attempts were made to solve the riddle of this selective breeding. It may be admitted at once that this riddle is far from solved, though the chemical and physical properties and the fauna and flora of breeding places have been rather intensively studied by various workers.

The early investigators, noting the absence of mosquito breeding in certain ponds, pools, etc., tried to offer explanations for such conditions. These explanations ran the gauntlet of a wide variety of surmises, usually observing that the water was unsuitable, that there was lack of the necessary food (though the food requirements were not then known and are not even at the present time), or that the excessive plant growth covered the surface of the water so as to prevent the larvae from obtaining air, or that filamentous algae were abundant in which the larvae became entangled and died or that numerous natural enemies, as predacious fishes, insects, etc., were present.

That aquatic plants may act as destroyers of living organisms was observed quite early. Mrs. Treat (1875) noted that the bladders of a *Utricularia* sp. contained many organisms, including insect larvae, and she tried to solve the mystery as to how these animals were caught and if the plant used them as food. Darwin (1875) in his "Insectivorous Plants" gives a much more detailed

account but failed to solve the mystery of how the organisms were trapped. Brocher (1911) fully solved the process which was later confirmed by Hegner (1926). Different workers in various parts of the world have called attention to this group of carnivorous plants and their possible utilization as agents in mosquito control.

Predacious Plants

The commonest species is probably *Utricularia vulgaris* (Fig. 1). It often occurs in great abundance floating directly below the surface of the water. The numerous small bladders and finely divided leaves distinguish it quite easily. The bladders are most interesting structures and have been fully described by Brocher and Hegner. These bladders, when "set" for the capture of prey, have their side walls rigidly compressed; organisms then enter the outer vestibule and by their movements stimulate the closing valve which suddenly opens,

Fig. 1. Portion of *Utricularia vulgaris*, showing the numerous bladders. Slightly magnified.

the side walls expand and the inrushing water carries the entrapped animal within. The valve then closes and the organism is firmly held. Within the bladders the animals are gradually digested and furnish food for the growth of the plants. The enormous amount of food taken by these plants may be illustrated by some of Hegner's observations. He found that the bladders on parts of a plant 220 cm (nearly seven feet) long contained approximately 150,000 small Crustaceans besides other organisms. From the standpoint of mosquito control the question may be asked do these bladders capture mosquito larvae and how many? Franca (1922) ob-

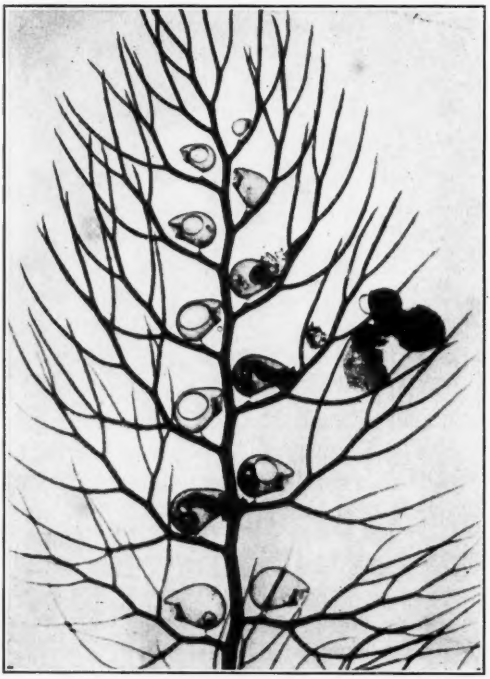

FIG. 2. A small portion of a branch of *Utricularia vulgaris*. Ten active bladders are shown and five of them contain mosquito larvae. Magnified 7 times.

served them capture numerous larvae of *Theobaldia longiareolate* and *Anopheles bifurcatus;* Brumpt (1925) records the capture of the larvae of *Anopheles maculipennis* and *Culex apicalis.* During the summer of 1929 I carried on a few experiments with this plant to determine the rate of destruction and the size of larvae they could capture. Fig. 2 shows a small branch containing ten active bladders. In five of these may be seen mosquito larvae in various stages of digestion. I have had them capture the smallest larvae and readily destroy the largest larvae I could obtain. Two preliminary experiments were conducted to determine the effectiveness of this plant in small aquaria. Two small branches were placed in a battery jar with fifty young larvae of *Culex territans.* On the following day only two free larvae could be found; the bladders contained the others. During the next four days 375 larvae were added and practically all were captured, though towards the end of the experiment nearly all the bladders had dropped from the branches. How long a single bladder may live and how often it can capture such large prey as mosquito larvae are not known. In another similar experiment 225 larvae were added and practically all these were captured before the bladders began falling from the plant.

In order to test the size of animals captured large larvae of *Brachydeutera argentata* Walk. (Ephydridae, Diptera) were added to an aquarium with Utricularia. The results are shown in Fig. 3. In cultures of Utricularia large numbers of these larvae were readily captured. Though only a small portion of the larva is first taken into the bladder our observations show that eventually the entire larva is absorbed. How this is done is not easily explained.

Though the captured food of the Utricularia would appear to be mainly small organisms belonging to the Crustacea, Protozoa, etc., yet it would seem that this group of plants deserves more consideration as a possible agent in mosquito control. The bladderworts are

FIG. 3. Small portions of branches of *Utricularia vulgaris* with bladders which have captured *Brachydeutera argentata*. Note the enormous size of the larvae as compared with the size of the bladders. Magnified about 7 times.

very graceful plants, grow luxuriantly where they occur and ought to make an added attraction in fish-ponds, small lakes, private pools, etc. Unfortunately we know very little about their biology or methods of their culture.

SURFACE-COVERING PLANTS

Another group of plants that has attracted considerable attention among students of the mosquitoes is the surface-covering aquatic plants. These plants are often packed so closely together that the entire surface is covered, causing the larvae to die from suffocation, or the plants in some way inhibit the female mosquitoes from laying their eggs in such situations. Smith (1910) investigated the reported effect of a species of *Azolla* growing on the water in the canals of Holland. Here he found it in certain regions, the peat and turf areas,

growing in such profusion as to cover the canals completely and prevent mosquito breeding. This plant seems to have restricted breeding areas, and though introduced into America its growth was not very successful. Conflicting reports as to its value have since been published, but as far as I know no experimental work has been undertaken to test its reported value. MacGregor (1920) thinks that *Azolla filiculoides* is a deterrent to anopheline breeding. He found no larvae or egg deposition in experimental tanks covered by this plant, whereas oviposition and breeding took place in nearby tanks containing other kinds of aquatic plants. Eugling (1921) states that the planting of *Azolla* in Albania seems to favor the breeding of anophelines. Mühlens (1924; 1925) reports that in parts of Argentina he found no mosquito larvae in pools and lagoons covered with *Azolla* sp. and *Azolla filiculoides,* whereas in nearby pools where these plants were absent mosquitoes bred in abundance.

Various species of the *Lemnaceae* (duckweeds) have been reported as effective water coverings (Fig. 4). In New Jersey, Johnson (1902) found that no mosquito breeding took place where the *Lemna* formed a complete mantle but where open spaces occurred *Culex* and *Anopheles* bred in small numbers. Furthermore *Lemna*-covered ponds harbor numerous predacious insects which attack mosquito larvae. Howard, Dyar and Knab (1913) state that one of the most abundant breeding grounds of *Culex salinarius* was a large marsh completely covered by *Lemna.* Bentley (1910) found that species of *Lemna* were of no value but that a related plant, *Wollfia arhiza* (see Fig. 4) was quite effective in the tanks in and around Bombay. In Sardinia, Fermi (1917) recommends the planting of *Lemna palustris* where oiling can not be employed. In Corsica, Regnault (1919) reports that *Lemna* was successfully grown and prevented mosquito breeding. Whenever the *Lemna* disappeared breeding

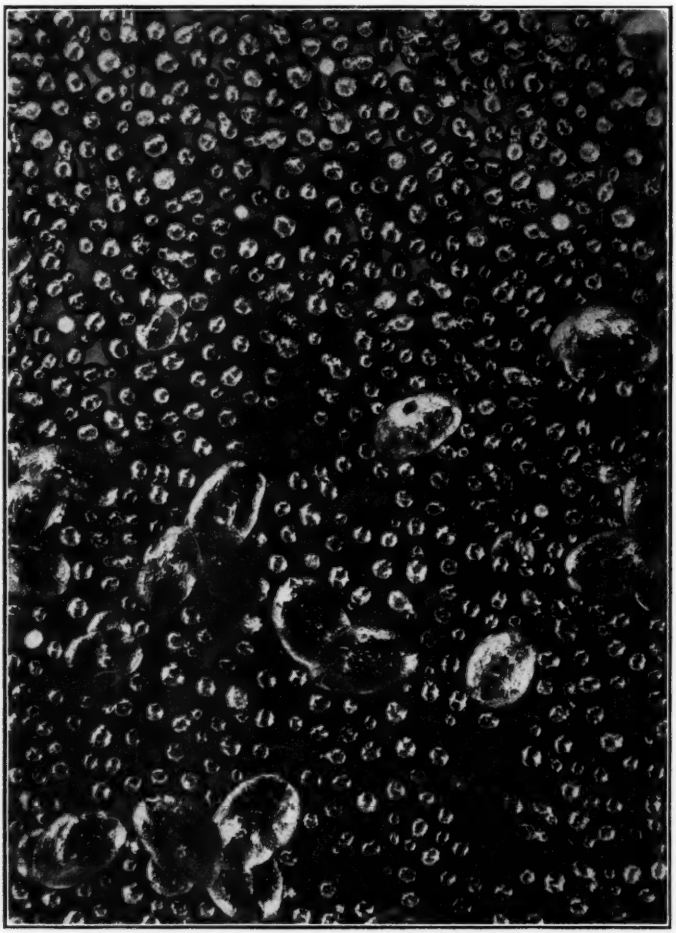

FIG. 4. Surface of water covered with *Wollfia punctata* and a few plants of *Lemna minor* (the larger plants). Magnified about 7 times.

took place. In Russia, Vasilev (1925) found that wherever *Lemna minor* and *Lemna polyrrhiza* covered the surface no mosquito larvae could be found. Other workers present very conflicting reports, but the evidence of general observations would seem to indicate that the various species of the *Lemnacae* may prove of value in reducing the abundance of mosquitoes. Fig. 4 shows an enlarged (magnified about seven times) view of *Wollfia punctata* and *Lemna minor* covering the surface of water. In ponds about Ithaca where these species grow in abundance few larvae are present. However, our knowledge of the growth or culture of these plants is very limited. A pond (Fig. 5) which for years past had a compact covering of *Lemna minor* suddenly in 1929 became densely coated with *Wollfia punctata*, the *Lemna* occurring only in small patches.

FIG. 5. A pond near Ithaca which for several years has been completely covered with *Lemna* sp. In 1929 the entire surface became suddenly covered with *Wollfia punctata*, a plant closely related to *Lemna*. Mosquito breeding has rarely been observed in this pool and then only scattered larvae.

From all these conflicting observations one fact stands out clearly—that the study of the growth, culture, distribution and usefulness of surface-loving plants deserves the especial attention of botanists and those engaged in the problems of mosquito control. This may be further emphasized by the report of Williamson (1928) that in Malaya the blue-green alga, *Microcystis*, forms a dense scum in foul waters and this accounts for the absence of anopheline larvae.

NON-SURFACE-LOVING AQUATIC PLANTS

Though predacious and surface-loving aquatic plants deserve more study, those graceful plants belonging to the *Characeæ* aroused the attention of mosquito workers when Cabellero (1919) announced that *Chara foetida* had a marked effect in inhibiting the development of mosquito larvae. This and his later studies awakened a renewed interest in the study of aquatic vegetation in relation to mosquito breeding. These studies have centered largely about the *Characeæ*, and numerous conflicting reports have been published in recent years. The various species of *Characeæ* (Fig. 6) are beautiful and graceful plants often growing with remarkable vigor and forming a most pleasing bottom covering for otherwise unsightly ponds. The species of *Characeæ* are very difficult to identify, and many workers seem to be of the opinion that certain species do inhibit larval growth while others have no effect. What are these species? This question has not yet been answered, for the experimental work with the various species has been very limited. Most of the published results deal with general field observations, and there is no indication by these various authors as to the exact conditions under which the observations were made. Was the *Chara* sp. growing vigorously? Were the ponds fouled with wastes? Were they temporary or permanent ponds? Were they spring fed, from overflows, surface water or from other sources? In other words, an exact analysis

FIG. 6. A few branches of *Chara fragilis* growing in an aquarium.

of the aquatic environment is seldom given. In general, such workers as Allaud (1922) in Morocco, Langeron (1921) in Tunis, Maynar (1923) and Pardo (1923) in Spain, Federici (1928) in Italy and Buhot (1927) in Australia have presented brief experimental data that seem to confirm the findings of Cabellero. On the other hand, the work of MacGregor (1924) in England, Barber (1924) in the United States, Fisher (1924) in Panama, Buxton (1924) in Palestine, Swellengrebel (1925) in Holland, Reyne (1924) in Surinam, Vasilev (1925) and Tarnogradski (1925) in Russia, and Hamlyn-Harris (1928; 1929) in Australia would indicate that the *Characeæ* have little, if any, effect on mosquito breeding. Blow (1924) reports little mosquito breeding in Madagascar where species of *Chara* abound, but in 1927 he states that the *Charophyta* (*Characeæ*) possess no larvicidal qualities. Hacker (1922) reports doubtful results in the Federated Malay States. Such conflicting results would indicate that we know very little of the under-

lying factors that induce or prevent mosquito breeding. Every worker is fully aware that certain types of aquatic situations induce mosquito breeding, whereas other or even almost identical situations do not bring about breeding. In other words, we have what may be called the selective breeding habits of each species. What are the factors that control or govern such selective breeding habits?

Early in 1923 I was impressed by such conditions in central New York and began a certain line of investigation in an attempt to answer some of these questions. The discovery of a permanent spring-fed pool (Fig. 7) richly carpeted with a growth of *Chara fragilis* enabled me to plan a line of work which might promise some results. In this pool, and similar ones since discovered, no mosquito breeding took place. It seemed ideal as a mosquito habitation. Farmhouses were located nearby and cattle grazed in large numbers in the surrounding

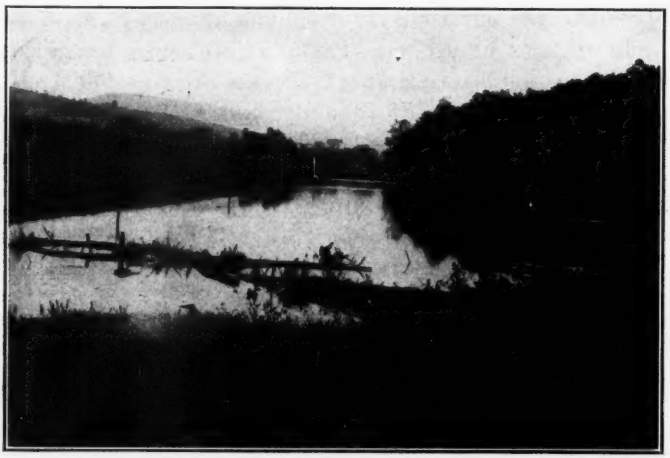

FIG. 7. A pool with a dense bottom covering of *Chara fragilis*. Along the margins and about the partially submerged logs would seem to be ideal places for mosquito breeding. No mosquito breeding has been found here for the past six years.

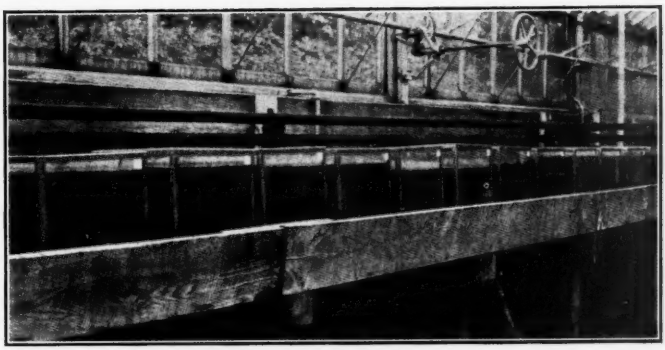

FIG. 8. A series of aquaria stocked with *Chara fragilis* to test the effect of this plant on mosquito larvae and on oviposition by the adults.

pastures so that the adults had a ready source of blood. Why, then, do mosquitoes fail to breed here? A few preliminary experiments made in 1925 seemed to indicate that the carpeting of *Chara fragilis* might be the inhibiting factor. Nothing further could be done till the spring of 1927. At that time I attempted to obtain answers to the following questions: (1) Does this species of *Chara* have an inhibiting effect on larval development? (2) Will mosquitoes oviposit readily on *Chara*-filled pools, and if they do what becomes of the larvae that hatch out? (3) If *Chara* has an inhibiting effect on the development of the larvae what is the causal agent? (4) Or, if the presence of *Chara* inhibits oviposition what are the factor or factors involved? (5) What is the food of mosquito larvae?

To answer the first question a long series of experiments was conducted in aquaria in our greenhouse (Fig. 8). The aquaria were stocked with *Chara fragilis*, and the larvae of various species of mosquitoes were added from time to time. Water from a nearby stream was used both in the *Chara* aquaria and in the controls. In the controls the larvae seemed to develop normally so whatever food was necessary was present in the water

added to the *Chara* aquaria. The results of these experiments are briefly summarized in Table I.[1]

TABLE I

RESULTS OF CHARA EXPERIMENTS

Species	No. larvae	Stages of larvae	Adults emerged	No. died	Time required
Aedes vexans	2500	Second to fourth	115	2385	2 to 14 days
Culex pipiens	4500	Second to fourth	379	4021	2 to 14 days
Culex territans	1100	Various	108	1092	2 to 15 days
Anopheles punctipennis	146	Various	8	138	3 to 25 days
Aedes canadensis	800	Young	223	577	4 to 10 days
Aedes stimulans	1250	Various	64	1186	6 to 17 days
Totals	10,296		897	9,399	

The table is self-explanatory. Not a large variety of species was tested in the experiments, but out of 10,296 larvae there was an emergence of only 897 adults. In the controls our emergence records show that nearly all the larvae produced adults. What, then, is the factor or factors that prevented these larvae from completing their development? I thought at first that the plant might produce some lethal substance, judging from the rapid death-rate of the larvae. This, however, seemed to be ruled out, as a great abundance of Cyclops, Daphnia and other small Crustacea as well as numerous phytoplankton thrived in the experimental aquaria. A factor experimented with was the pH values (acidity *vs.* alkalinity). The experimental aquaria and our *Chara* ponds showed a wide daily cycle of pH values, usually running from a pH of 7.6 to nearly 9.4 each day. The water was always alkaline and following MacGregor (1921) and Senior-White (1926) I thought the changing

[1] Full details may be found in the *American Journal of Hygiene*, 8: 279–292, 1928.

alkalinity might account for the high death-rate. Further experiments confirmed the results of other workers that pH values have probably little to do with inhibiting larval development. The next factor was the study of the food requirements of the larvae. Could it be possible that *Chara* ponds and our *Chara* aquaria did not possess sufficient food for the development of the larvae?

An intensive search of the literature showed that the main larval food consists of the zooplankton and phytoplankton organisms found in the water. These are swept in by the action of the larval mouthbrushes, and everything that can be swallowed is taken in indiscriminately. Along with these substances is swept in a considerable amount of water. Fortunately I had begun an intensive study of the plankton of a typical mosquito breeding pool and the spring-fed *Chara* pond. This investigation[2] soon showed a greater variety and a much higher density of plankton organisms in our *Chara* pond and aquaria than that found in a typical mosquito breeding pool. If plankton organisms are the food of mosquito larvae here then was an abundant food supply and yet they died amidst plenty. However, I have never fully believed that plankton organisms constitute the entire larval food but that the larvae probably obtain most of their food from substances in solution in the water and from decaying organic wastes. My assistant was in the midst of testing this theory, and his preliminary results indicated that my thesis held true for the species with which he was experimenting. If plankton does not constitute the main food of mosquito larvae but rather substances in solution and organic wastes, then the question is does the *Chara* remove these substances in solution so rapidly as to starve the larvae? Decaying organic matter is largely absent in *Chara* ponds, as one of their marked characteristics is the crystalline clarity of

[2] The results are to be published in detail in a forthcoming number of the *American Journal of Hygiene*.

the water. I could find no way to test all these suppositions experimentally.

There was still another factor which always excited my curiosity, more especially since the publication of Cleveland's work in defaunating the intestinal tract of termites with oxygen. As *Chara* gives off during the day an immense number of tiny bubbles of oxygen I felt fully convinced that the larvae must sweep hundreds of them into their intestines. What effect could the presence of oxygen have on the digestive processes of the larvae? To test this I devised a rather simple apparatus. Pure oxygen was passed, under pressure, through Berkefeld filters N and W and entered the water in bubbles almost as tiny as those given off by the *Chara*. Fig. 9 shows the apparatus in operation. Two cylinders are being treated with oxygen while two others serve as controls. The same water and food were supplied to all the jars. This experiment was conducted for a considerable time and a brief summary is presented in Table II.

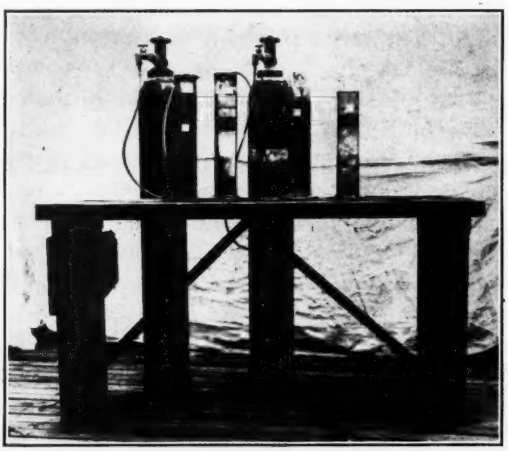

FIG. 9. Apparatus for testing the effect of oxygen on larvae. Two cylinders are being treated while two others serve as controls.

TABLE II

TESTS WITH OXYGEN

Species	No. larvae	No. died	No. adults	Time exposed	Controls
Culex sp.	50	50	0	3 days	Adults emerged 2 days later.
"	50	50	0	7 days	Adults emerged as experiment ended.
"	50	50	0	3 days	Adults emerged a few days later.
"	50	50	0	4 days	Adults emerged a few days later.
Anopheles punctipennis	20	?	A few	9 days	Adults all emerged in controls.

These experiments and many others which were tried indicate that the oxygen present in the water in small bubbles may be ingested by the larvae and may bring about a high death-rate. The larvae in the oxygen-treated cylinders appeared irritable, and though their intestines were filled with apparently normal food yet their growth was slow and they gradually dropped to the bottom and died. There was great difficulty in preventing the oxygen bubbles from accumulating at the surface. Whether or not larvae could break through the surface film with their air-tubes remains unsolved. This may prove the real reason for the death of the larvae. However, the presence of the oxygen in the intestines (not proved, except by inference) may interfere with the digestive functions and bring about the death of the larvae. It may be recalled that Cleveland (1925) brought about the death of termites by defaunating their intestinal tracts by means of oxygen. In this case the oxygen killed the commensals (Protozoa) which converted the eaten wood into substances capable of being digested by the termites. What oxygen may do in the intestinal tracts of mosquito larvae remains to be solved.

In addition to the aquaria experiments with *Chara* a long series of tests was conducted with wooden tubs sunk

FIG. 10. A series of wooden tubs sunk in the ground to test the effect of *Chara fragilis* under out-door conditions. Some of these tubs are used as controls.

in the ground (Fig. 10), with wooden tanks divided into compartments (Fig. 11–3) so that there would be *Chara* growths separated from the ordinary water by screens, and also to provide a method of testing *Chara* in still as compared with running water. The results of these experiments[3] were uniformly successful and gave additional evidence that *Chara fragilis* in some way prevented the development of the larvae.

The second problem, do females normally oviposit in pools containing vigorous growths of *Chara,* was also tested experimentally. A series of aquaria filled with a growth of *Chara fragilis* was set up in our greenhouse (Fig. 8). An abundant supply of the adults of two *Culex* species (*C. pipiens* and *C. territans*), *Anopheles punctipennis,* and *Aedes aegypti* (the yellow fever mosquito) was at all times present in and about our experi-

[3] A full account of these experiments may be found in the *American Journal of Tropical Medicine,* 9: 249–266, 1922.

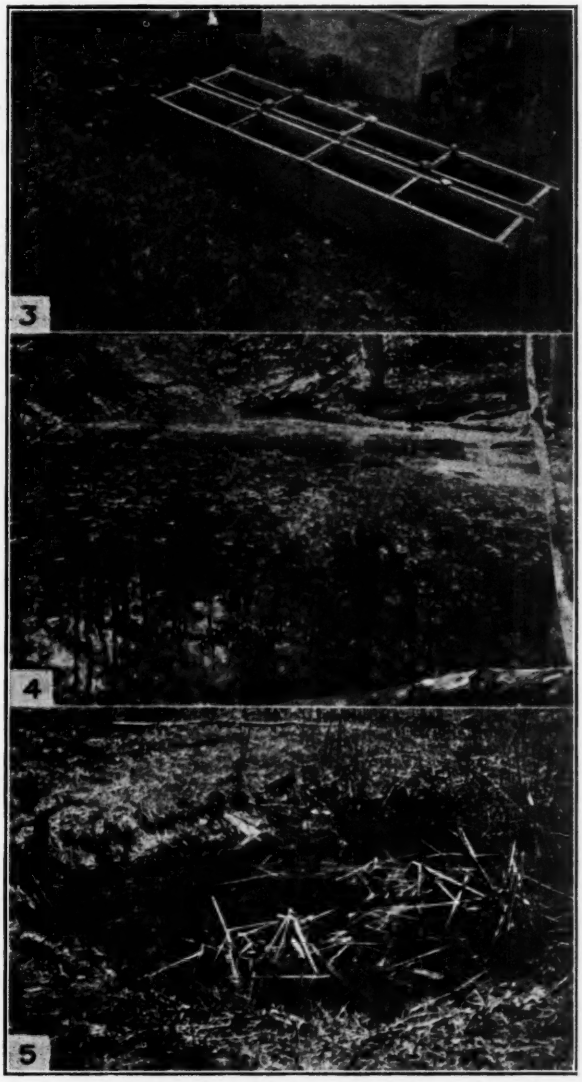

FIG. 11. (3) Wooden tanks divided into compartments to test *Chara fragilis* in still and running water. (4) A deep woodland pool in which *Chara fragilis* was introduced. (5) A spring-fed pool (Buttermilk Falls Pool) planted with *Chara fragilis*.

mental quarters. The results of these experiments are shown in Table III.

TABLE III

AQUARIA EXPERIMENTS WITH *Chara fragilis* TO TEST EGG DEPOSITION

Experiment number	Time set up	Maximum pH	Condition of *Chara*	Results
1052–21	June 25	9.5	Vigorous till July 26. Decay till August 23. Vigorous thereafter	No egg masses till July 29 (4), 30 (2). Larvae matured. No more egg masses during season.
1052–22	June 19	9.5	Vigorous growth all season (October 24)	No egg deposition all season. 65 larvae *C. apicalis* added (July 14). All died.
1052–23	June 19	9.5	Much decay till July 9. Vigorous thereafter	Egg deposition 1 (June 27), 1 (July 2). Larvae from first matured, others died. No further egg deposition.
1052–24	June 19	9.5	Maintained vigorous growth all season	No egg deposition throughout the season.
1052–25	June 19	9.5	Much decay till July 9. Vigorous growth thereafter	1 egg mass (June 26), larvae matured; 1 (July 2), hatched? No more egg masses.
1052–28	June 19		*Chara* died down (July 21). Replaced, died (July 30)	Egg deposition took place all through the season and many adults emerged.
1052–29	June 19	9.5	*Chara* did not become vigorous till July 11. Vigorous thereafter	Egg masses 1 (June 27), 1 (June 28), 2 (July 2), 1 (July 5). No more during season. Many larvae failed to mature.
1052–42	August 2	9.2	*Chara* began to decay August 4 and completely died down	*Culex* egg masses and larvae through the season. Abundant till September 25.

An examination of the table brings out a most important fact—that wherever there is decay and dying of the *Chara* oviposition by *Culex* species took place almost immediately. In experiments 1052–22 and 24 the *Chara* maintained a vigorous growth throughout the season and no oviposition took place. In those experiments in which considerable decay took place at the beginning oviposition is recorded and most of the larvae which hatched from the first egg masses matured. Those that hatched from egg masses deposited when the *Chara* was regaining vigor failed, in most cases, to mature. In

those aquaria in which growth did not take place (1052–28, 42, 43, 44 and 45) oviposition was excessive throughout the season and immense numbers of adults emerged. Yet despite this density of adults no oviposition took place in those aquaria containing a vigorous growth of *Chara*. Why mosquitoes do not oviposit on water containing a vigorous growth is not known. It may be added that no oviposition took place in our outdoor tubs or wooden troughs where the *Chara* growth was vigorous. Oviposition always began when decay started and became excessive as the decay increased.

These and other laboratory experiments appeared so promising that a survey of the local area was undertaken to discover if pools, ponds, lakes, etc., where *Chara* flourished were free from mosquito breeding. Numerous more or less isolated ponds were found with vigorous growths of *Chara* and in practically every case mosquito breeding did not occur. These results were so encouraging that attempts were made during the seasons of 1928 and 1929 to introduce *Chara fragilis* into as wide a variety of known breeding places as possible. This was done and several of these introductions were studied during 1928 and 1929. In order to understand the problems of *Chara* introductions the breeding habits of our northern species of mosquitoes must be borne in mind. All our *Aedes* species pass the winter in the egg stage, the eggs being deposited throughout the summer on the bottoms or margins of dried out or greatly lowered pools. These eggs, with the possible exception of *Aedes vexans,* do not hatch till the following spring. All our species of *Culex, Anopheles* and most of our *Theobaldia* pass the winter as adults and oviposit on water during the following spring or summer. It will thus be seen that we have practically only one brood of the *Aedes* species each season, whereas there may be several broods of *Culex, Anopheles* or *Theobaldia* species. There are exceptions to this general summary, but the main thesis holds true for the species under experimentation with

Chara. In any attempt to introduce *Chara* into pools which dry up during the summer the question of the renewal of growth the following season was problematical. However, for a number of years *Chara fragilis* was found growing in temporary puddles which dried out each season, so it was thought introductions into similar pools might prove successful. Furthermore, the kind of water which would support *Chara* was not known and information on the culture, growth, etc., of *Chara* species appeared practically nil.

It may be worth while to present the details of two introductions.

Buttermilk Falls Pool.—This pool (Fig. 11–5) is rather small and is spring fed. It is about eight feet in diameter and two and a half to three feet deep. *Aedes canadensis* breeds here in great abundance during the early spring and is followed by *Aedes vexans, Culex apicalis, Culex territans* and *Anopheles punctipennis.* The main object of the experiment was to obtain a good growth of the *Chara* which might prevent the breeding of the summer species and also prevent the oviposition of *Aedes canadensis* and *Aedes vexans.* The results of this experiment are shown in Table IV.

From Table IV it will be observed that the *Chara* was added on April 9, 1928. At that time larvae of *Aedes canadensis* were very abundant. Growth of the *Chara* did not begin till about May 13 when the first pupae appeared. The development of the larvae was undoubtedly retarded, for pupation took place in nearby pools a week earlier. From June 7 till July 3 there was no breeding in this pool except a few larvae on June 7. In the nearby pools *Aedes canadensis, Culex territans* and *Culex apicalis* were present in both the larval and pupal stages. Early in July the nearby pools became dry, and from then till September breeding took place in our experimental pool. It should be noted, however, that early in July (July 3) a considerable area of decay appeared and this continued throughout the season. Breeding began

TABLE IV

BUTTERMILK FALLS POOL

Date	Condition of *Chara*	Results
April 7, 1928		Numerous larvae of *Aedes canadensis*
April 9	Introduced 4 pails of *Chara*	Larvae very inactive, due to cold
April 18	Not growing	Larvae abundant, growth slow
May 2	Not growing	Larvae abundant, growth slow
May 13	Growth begins	Larvae abundant; a few pupae
May 17	Good growth	Pupae numerous
May 31	Good growth	*Anopheles punctipennis*, *Culex apicalis* } A few small larvae
June 7	Slow growth	*Anopheles punctipennis*, 2 large larvae. *Culex apicalis*, a few small ones
June 13	Vigorous growth	No breeding; larvae and pupae in nearby pools
June 16 to 28	Vigorous growth	No breeding; nearby pools dry
July 3	Good growth, but decay area in center	*Culex apicalis*, few small larvae; *Anopheles*, a few larvae
July 5 to September 6	Vigorous growth, but a central area of marked decay	*Culex apicalis* and *Anopheles punctipennis* bred in fair numbers throughout this period.
1929		
March 25	No *Chara* growth	A few young larvae, *Aedes canadensis;* nearby pools swarming with this species
April 2	" " "	Larvae very scarce; *A. canadensis* and *A. excrucians*
April 20	" " "	Pool high; larvae very scarce; 50 obtained after much difficulty
May 1	" " "	A few large larvae of *A. canadensis*
May 11	" " "	A few small larvae present
May 23	" " "	A few small *A. canadensis;* nearby pools with numerous large larvae and pupae
May 30	" " "	A few large larvae. In nearby pools adults had emerged.
June 13	" " "	No larvae present; nearby pools practically dry
June 18	" " "	A few larvae of *Culex apicalis* and two of *Anopheles punctipennis* found; nearby pools dry
July 1	" " "	No breeding; nearby pools dry
July 16	" " "	No breeding; nearby pools dry
August 2*	" " "	No breeding; nearby pools dry

* During the rest of the season there was no growth of the *Chara* and no breeding took place.

with the appearance of decay and this result agrees with that obtained in our experimental aquaria. Another point might be noted: *Aedes vexans* bred in nearby pools but was never found in the experimental pool. The results for 1929 are not very encouraging. The *Chara* did not apparently survive the winter. It is hoped that growth may again show up in 1930. In 1928 the larval density of *Aedes canadensis* was most extraordinary. A few sweeps of a small net brought in nearly 2,000 larvae, and immense numbers of adults emerged. In 1929 it will be seen we had difficulty in obtaining even 50 larvae. Whether this reduction in breeding was due to the *Chara* preventing oviposition in 1928 is difficult to say. Had the *Chara* continued to grow in 1929 we might be justified in concluding that *Aedes canadensis* refused to oviposit where *Chara* grows. However, as compared to nearby pools there was a reduction of mosquito breeding in 1928 and an even more marked reduction in 1929. The nearby pools swarmed with *Aedes canadensis,* whereas the *Chara* pool contained an extremely small number of larvae.

The woodland pool.—The woodland pool (Fig. 11–4) is a large pot-hole which usually maintains a water supply till late August or September. Here breed *Aedes stimulans, A. excrucians, A. fitchii* and *A. punctor.* The larval density is usually very high, and immense numbers of adults swarm during the summer in the surrounding woodlands. Nearby are numerous breeding places for these species so that if *Chara* would grow and maintain itself in this pot-hole we would have an excellent opportunity to test its efficiency. The introductions were made on April 14 and 21, 1928. During the summer of 1928 the *Chara* maintained a considerable growth, but in 1929 none could be found. Though the experimental pool had a high larval density in 1928 there were very few larvae in 1929. Unfortunately the nearby pot-holes where there was a high larval density in 1928 had comparatively few in 1929. In fact, this whole wood-

land area, which usually swarmed with mosquitoes during the summer, had very few during the season of 1929. The reduction was probably due to a salamander (*Diemictylus viridescens*) which swarmed in all the pools during 1928 and 1929. This salamander has been shown to be a voracious feeder on mosquito larvae.

Though numerous other introductions were made, none of them could be followed up in detail. Several new introductions were made in 1929, and I have hopes that some of these may prove more successful. Our failures are due, in all probability, to our ignorance about the biology and cultivation of the *Characeæ*. Though I have worked with only one species, *Chara fragilis,* other investigators have employed several other species sometimes with apparent success but unfortunately more often with failure. However, this line of work is directing more and more students to study the underlying factors of larval food, selective breeding habits of mosquitoes and the water conditions which induce or prevent mosquito breeding.

Other Aquatic Plants

Little experimental work has been done with other aquatic plants. Zetek (1920) reports that in the Panama Canal Zone anophelines were found breeding in the floating islands and other masses of water lettuce (*Pistia stratiotes*). In Brazil Bachmann (1921) found that mosquitoes appear to avoid *Pistia stratiotes, Myriophyllium brasiliense* and *Lemna.* He states that *Myriophyllium brasiliense* is being planted along the streams at points where breeding places have been cleared away. In the southern United States Barber and Hayne (1925) find that anophelines breed amidst water hyacinth (*Piaropis crassipes*).

I did some work with another aquatic plant, *Elodea* (*Phyllotria*) *canadensis* (Fig. 12). For a number of years this plant has grown in abundance in pools along a railway embankment. The pools seemed ideal places

FIG. 12. A small portion of *Elodea* (*Phyllotria*) *canadensis* growing in an aquarium. Slightly magnified.

for mosquito breeding, yet larvae were rarely found. Three experimental aquaria were run under similar conditions to those used with *Chara*. The results are shown in Table V.

TABLE V

EXPERIMENTS WITH *Elodea* (*Phyllotria*) *canadensis*

Date	Aquarium 1052–33	pH	Aquarium 1052–34	pH	Aquarium 1052–35	pH
July 10	Set up		Set up		Set up	
July 12	Vigorous growth	9.1	Vigorous	9.2	Vigorous	9.1
July 16	+200 *Culex* larvae, various	9.3	+200 *Culex* larvae, various	9.3	Vigorous	9.3
July 18, 19	16 adults	9.3	22 adults	9.4	+200 *Aedes vexans*, small	9.4
July 31	+200 *Culex* small	9.4	+200 *Culex* small	9.4	No larvae ?	9.4
August 9	No larvae, no adults	9.3	No larvae, no adults	9.4	No larvae, no adults	9.4

This table is self-explanatory. How are we going to account for the high death-rate? Furthermore, these aquaria were exposed practically the entire summer to the large numbers of adult mosquitoes that were constantly emerging from our other aquaria, yet in not a single instance did we find an egg mass deposited on them. I have no explanation to offer for the results. If the experiments with oxygen can be successfully repeated with more species of mosquitoes I would surmise that the excessive amount of oxygen given off in minute bubbles by this plant may oxidize the organic wastes too rapidly, destroy any foods in solution or, when ingested by the larvae, interfere with their digestive processes.

The whole problem of selective mosquito breeding involves a fundamental study of aquatic environments. Water which is everywhere so common and abundant, a substance essential to all life, a fundamental necessity to every life process, is one of the most puzzling substances known. Though much has been attempted little fundamental knowledge of water chemistry, of the physicochemical factors of water, of water solutes and their exact constitution or reactions, etc., has been gained. Until we know something more fundamental about the physicochemical factors involved in the relationship of water to life processes we can scarcely hope to make much progress in interpreting the aquatic environments of living plants and animals.

LITERATURE CITED

Allaud, C.
1922. "Rapport à Monsieur le Directeur Général des services de Sante du Maroc sur une Mission antipaludique," *Bull. Soc. Sci. Nat. Maroc.*, 2: 3–6.

Bachmann, A.
1921. "Programa de Ducha para Llevarse á Cabo en Famailla contra los Anofeles y sus Larvas," *Anales Dept. Nac. Higiena,* Buenos Aires, 27: 117–137.

Bauer, J. H.
1928. "The Transmission of Yellow Fever by Mosquitoes other than *Aedes aegypti*," *Amer. Jl. Trop. Med.*, 8: 261–282.

Barber, M. A., and Hayne, T. B.
1925. "Water Hyacinth and the Breeding of Anopheles," U. S. Pub. Health Rept., 40: 2557–2562.

Barber, M. A.
1924. "The Effect of *Chara robinsii* on Mosquito Larvae," U. S. Pub. Health Rept., 39: 611–615.

Bentley, C. A.
1910. "Natural History of Bombay Malaria," *Jl. Bombay Nat. Hist. Soc.*, 20, 392–422.

Blow, T. B.
1924. "Notes on Charophyta in Madagascar," *Jl. Botany*, 62: 252–253.
1927. "Observations on the Alleged Larvicidal Qualities of the Charophyta," *Proc. Linn. Soc. London*, Session 139, 46–47.

Brocher, F.
1911. "Le Problème de l'Utriculaire," *Ann. de Biol. Lacustre*, 5: 33–46.
1927. "A propos de la capture de larves d'Anopheles par les Utriculaires," *Ann. Parasit. hum. et comp.*, 5: 46–47.

Brumpt, E.
1925. "Capture des Larves de Culicides par les Plantes du genre Utricularia," *Ann. Parasit. hum. et comp.*, 3: 403–411.

Buhot, E. W. I.
1927. "Effects on Mosquito Larvae of a Queensland Nitella," *Proc. Roy. Soc. Queensland*, 38: 59–61.

Buxton, P. A.
1924. "Applied Entomology of Palestine, Being a Report to the Palestine Government," *Bull. Ent. Res.*, 14: 289–340.

Cabellero, A.
1919. "La Chara foetida A Br., y las Larvas de Stegomyia, Culex y Anopheles," *Bol. R. Soc. Española Hist. Nat. Madrid*, 19: 449–455.

Cleveland, L. R.
1925. "The Effects of Oxygenation and Starvation on the Symbiosis between the Termite Termopsis and its Intestinal Flagellates," *Biol. Bull.*, 48: 309–326.

Darwin, Charles.
1875. "Insectivorous Plants."

Eugling, M.
1921. "Über Malariabekampfung," *Beihefte Arch. Schiffs-u. Trop.-Hyg.*, Leipzig, 25, No. 1, 63 pp.

Federici, E.
1928. "L'azione tossica delle 'Charae' sulle larve dei Culicidi," *Redia*, 16: 18–28.

Fermi, C.
1917. "La Profilassi antimalarica in due Citta sarde," *Annali d'Igiene*, 27: 228–236. Rome.

Fisher, H. C.
1924. "Report of the Health Department of the Panama Canal Zone for 1922," Mount Hope, C. Z., 1923. Also in Report for 1923.

França, C.
1922. "Recherches sur les plantes carnivores. II. *Utricularia vulgaris*," *Bol. Soc. Broteriana*, I (2nd. Ser.) part 1: 11–37. Coimbra.

Hacker, H. P.
1922. "Malaria Bureau Annual Report."
1923. "Fed. Mal. States, Med. Rept.," 1922, Suppmt. to *F. M. S. Govt. Gaz.*, 27: 16–22.

Hamlyn-Harris, R.
1928. "The Relation of Certain Algae to Breeding Places of Mosquitoes in Queensland," *Bull. Ent. Res.*, 18: 377–389.
1929. "The Relative Value of Larval Destructors and the Part They Play in Mosquito Control in Queensland," *Proc. Roy. Soc. Queensland*, 41: 23–38.

Hegner, R. W.
1926. "The Interrelations of Protozoa and the Urticles of Utricularia," *Biol. Bull.*, 50: 239–270.

Howard, L. O., Dyar, H. G., and Knab, F.
1913. "The Mosquitoes of North and Central America and the West Indies," Vol. 1.

Johnson, H. P.
1902. "A Study of Certain Mosquitoes in New Jersey and a Statement of the Mosquito-malaria-theory," Ann. Rept., N. J. Agr. Exp. Sta.

Langeron, M.
1921. "Deuxième mission parasitologique en Tunisie, Tamerga," *Arch. Inst. Pasteur Afr. Nord.*, Tunis, 1: 347–382.

MacGregor, M. E.
1920. "The Possible Use of *Azolla filiculoides* as a Deterrent to Anopheline Breeding," *Jl. R. A. M. C.*, 34: 370–372.
1921. "The Influence of the Hydrogen-ion Concentration in the Development of Mosquito Larvae," *Parasitology*, 13: 348–351.
1924. "Tests with *Chara foetida* and *C. hispida* on the Development of Mosquito Larvae," *Parasitology*, 15: 382–387.

Matheson, R., and Hinman, E. H.
1928. "*Chara fragilis* and Mosquito Development," *Amer. Jl. Hygiene*, 8: 279–292.
1929. "Further Studies on *Chara* spp. and Other Aquatic Plants in Relation to Mosquito Breeding, *Amer. Jl. Trop. Med.*, 9: 249–266.

Maynar, J.
1923. "Contribution al studio de la accion larvicida de las Caraceas," *Bol. R. Soc. Española Hist. Nat.*, 23: 389–392.

Mühlens, P., Dios, R. L., Petrocchi, J., and Zuccarini, J. A.
1925. "Estudios sobre el Paludismo y Hematologia en el Argentino," *Rev. Inst. Bact.*, 4: 207–357.

Mühlens, P.
1924. "Informe preliminar sobre estudios realizados en los territorios del Chaco y Formosa y en el Paraguay," *Ann. Dept. Nac. Higiena*, 30: 139–146.

Pardo, L.
1923. "Observaciones acerca de la accion de la Chara sobre les larvas de los mosquitos," *Bol. R. Soc. Española Hist. Nat.*, 23: 154–157.

Regnault, F.
1919. "La culture des lentilles d'eau dans la lutte contra le paludisme," *Bull. Soc. Path. Exot.*, 12: 735–736.

Reyne, A.
1924. "Eenige proeven met vischjes en chemicalien tot het dooden van muskietenlarven," Dept. Landbouw, Nijverheid en Handel in Surinam, *Bull.*, 47, 54 pp., Paramaribo.

Senior-White, R.
1926. "Physical Factors in Mosquito Ecology," *Bull. Ent. Res.*, 16: 187–248.

Smith, J. B.
1910. "Azolla *vs.* Mosquitoes," *Ent. News*, 21: 437–441.

Swellengrebel, N. H.
1925. "A Summary of the More Important Facts on Adult Anophelines and Their Larvae, Observed by Us or Brought to Our Notice during our Tour through Eastern Europe and Italy (May–September, 1924)," Fol., 19 pp. Geneva, Soc. des Nations, May, 1925.

Tarnogradski, D. A.
1925. "Notes on the Biology of Mosquitoes," (in Russian), summary in *Rev. App. Ent.*, Ser. B., 13: 161.

Treat, Mary
1875. "Plants that Eat Animals," *The Gardener's Chronicle*, n. s., 3: 303–304.

Vasilev, I. V.
1925. "On the Biology and Ecology of the Common Malaria Mosquito, *Anopheles maculipennis*" (in Russian), summary in *Rev. Appl. Ent.*, Ser. B., 13: 161.

Williamson, K. B.
1928. "Mosquito Breeding and Malaria in Relation to the Nitrogen Cycle," *Bull. Ent. Res.*, 18: 433–439.

Zetek, J.
1920. "Anopheles Breeding among Water Lettuce. A New Habitat," *Bull. Ent. Res.*, 11: 73–75.

SHORTER ARTICLES AND DISCUSSION

A NOTE ON FISHER'S THEORY OF THE ORIGIN OF DOMINANCE, AND ON A CORRELATION BETWEEN DOMINANCE AND LINKAGE

R. A. FISHER (28 a, b) has suggested that, on its first appearance, a mutant gene usually produces a marked effect in the heterozygous condition, and becomes recessive to the wild type only as the result of natural selection acting over very long periods. He regards this process as due to the selection of specific modifying genes which render the appearance and viability of the heterozygote approximately the same as those of the wild type. S. Wright (29) has criticized this process on the ground of its slowness. The present note raises the question of whether it is possible in the manner suggested by Fisher.

Consider, with Wright, a population mostly of composition AAmm, and that Aamm have a viability $1-k$, compared with the proportion of Aa in the population is $\frac{2u}{k}$ (Haldane, 27a). Next consider a modifier M, which increases the viability of Aa to normal (the most favorable case for Fisher's theory). It is clear that AAMm can not have a viability greater than normal, or M would spread through the population apart from its modifying effect. Hence its presence in the species would have nothing to do with the mutability of A. Suppose the viability of AAMm to be $1-k'$, then it carries a disadvantage $-k'$ in most cases, an advantage $+k$ in a fraction $\frac{2u}{k}$ of all cases; *i.e.*, when combined with Aa. If it is to spread, $\frac{2u}{k} \cdot k > k'$, *i.e.*, $k' < 2u$. If we take u as 10^{-6}, the viability of AAMm must lie between .999998 and 1.0. Now suppose a number of M genes exist which raise the viability of Aa nearly to normal. I suspect that the viabilities of the AAMm zygotes will be fairly evenly distributed over values between 1.0 and .99, even if lower viabilities are rarer. If so, the chances are over 5,000 to 1 against any given one of them being selected as a result of the Fisher effect.

If an infinite number of possible modifiers were available, this would not matter, but the number of loci at which mutation can occur is finite. Hence I conclude that it is only in rare cases that

suitable modifiers will be found. If, however, they do exist, then owing to the very nearly normal viability of AAMm they will be fairly common in the population as the result of mutation, and will spread somewhat more rapidly than would otherwise be the case. The fact that modifiers rapidly appear in an inbred mutant race does not dispose of this argument, unless it can be shown that these modifiers have no adverse effect on the viability of the wild type.

I suspect that the Fisher effect has operated in a different manner. Adopting Goldschmidt's (27) view that genes are catalysts acting at a definite rate, there is, as pointed out elsewhere (Haldane, 27b) no obvious way of distinguishing those which act at more than a certain rate. *E.g.*, if an enzyme can oxidize a certain substance as quickly as it is formed, no visible result arises from doubling the amount of that enzyme. Hence, while a minus mutation (diminution of activity) of a normal gene may yield a recessive type, a plus mutation is often unobservable. Now on this hypothesis we have to explain why a wild-type gene generally has a factor of safety of at least 2, as is shown by the fact that one wild-type gene has nearly the same effect as two. If we imagine a race whose genes were only just doing the work required of them, then any inactivation of one of a pair of genes would lead to a loss of total activity. Thus if A_1A_1 can just oxidize all of a certain substrate as fast as it is formed, its inactivation will produce a zygote A_1a which can only oxidize about half. If now A_1 mutates to A_2, which can oxidize at twice or thrice the rate of A_1, if necessary, no effect will be produced, *i.e.*, A_1A_2 and A_2A_2 zygotes will be indistinguishable from A_1A_1. But A_2a will be normal. Hence A_2a zygotes will have a better chance of survival than A_1a, and A_2 will be selected.

In other words the modifiers postulated by Fisher are probably the normal allelomorphs of mutant genes, and the Fisher effect is rather to accentuate the activity of genes already present than to call up new modifiers. This hypothesis would not, of course, explain the behavior of the "crinkled dwarf" type of Sea Island cotton, which behaves as a recessive within the species, but gives intermediates in F_1 and F_2 with other New World cottons. Now the haploid number in these cottons is 26, as opposed to 13 in Old World cottons. They are therefore tetraploids, and failures of Mendelian inheritance are to be expected on crossing them. Several equally plausible hypotheses as to their genetical nature would explain the results cited by Fisher.

No theory of dominance can be complete which does not take cognizance of the fact that there are certain organisms in which mutant types are generally dominant, and not recessive to the normal type. For example, Nabours (17, 25) found eleven "genes" dominant to the most frequently occurring type of *Apotettix eurycephalus,* and none recessive; fourteen dominant and no recessive in *Paratettix texanus.* Similarly Winge (27) found eighteen "genes" dominant to the normal and none recessive in *Lebistes reticulatus.* A few other cases, less completely studied, fall into the same class. Now these organisms all share another peculiarity, namely, that the number of linkage groups is much smaller than the number of chromosomes. *Apotettix* has one autosomal linkage group, with seven pairs of chromosomes, *Paratettix* three linkage groups with six or seven pairs of chromosomes. *Lebistes* has twenty-three pairs of chromosomes, with one autosomal "gene" and the other seventeen sex-linked.

Haldane (20) and Demereč (28) have suggested that these results are due to linkage between chromosomes. A study of Nabours' (25) linkage map for *Apotettix* suggests the possibility that his eleven "genes" lie in four or five chromosomes, and that crossing over within a chromosome is extremely rare. How are we to explain the correlation between dominance of mutant types and excessive linkage? My friends Mr. C. D. Darlington and Mr. C. L. Huskins have suggested that these dominant mutant types differ from the normal, not by single genes, but by duplication or translocation of whole sections of chromosome. Translocation will account for linkage between chromosomes, on the lines of the theory developed by Darlington (29) for *Oenothera.* Thus both features can be explained if the chromosomes have an unusual tendency to break up and reunite in novel ways.

The cytological facts known in other Orthoptera and notably the work of Carothers (17, 21) support this view. Differences which are probably translocations or duplications have been seen, and behave in a Mendelian manner. There is no obvious ring formation, but it must be remembered that in *Oenothera,* where this occurs, interchromosomal linkage is nearly, if not quite, absolute. The rarity of crossing over within a chromosome is readily explained by the postulated differences between nearly homologous chromosomes.

Now on Fisher's theory there is no obvious reason why modifiers should not be able to suppress the effect of a duplication as easily as that of a gene. On the theory here adopted we need

only suppose that some of the genes in the duplication have a greater effect when three or four are present than when two only are found, as in the normal type.

To sum up, it is suggested that dominance may be due to either of three causes: the Fisher effect in rare cases, the overactivity of normal genes due to a modified Fisher effect in most cases and duplication of a section of chromosome in still a third small group.

J. B. S. HALDANE

JOHN INNES HORTICULTURAL INSTITUTION,
MERTON, ENGLAND

LITERATURE CITED

Carothers, E. E.
1917. "The Segregation and Recombination of Homologous Chromosomes as Found in Two Genera of *Acrididae*," *Journ. Morph.*, 28: 445–521.
1921. "Genetical Behavior of Heteromorphic Homologous Chromosomes of *Circotettix* (Orthoptera)," *Journ. Morph.*, 35: 457–483.

Darlington, C. D.
1929. "Ring-formation in *Oenothera* and other Genera," *Journ Gen.*, 20: 345–365.

Demerec, M.
1928. "A Possible Explanation for Winge's Findings in *Lebistes reticulatus*," AMER. NAT., 62: 90–94.

Fisher, R. A.
1928a. "The Possible Modifications of the Responses of the Wild Type to Recurrent Mutations," AMER. NAT., 62: 115–126.
1928b. "Two Further Notes on the Origin of Dominance," AMER. NAT., 62: 571–574.

Goldschmidt, R.
1927. "Physiologische Theorie der Vererbung."

Haldane, J. B. S.
1920. "Note on a Case of Linkage in *Paratettix*," *Journ. Gen.*, 10: 47–52.
1927a. "A Mathematical Theory of Natural and Artificial Selection, Pt. V," *Proc. Cam. Phil. Soc.*, 23: 838.
1927b. "The Comparative Genetics of Color in Rodents and Carnivores," *Biol. Rev.*, 2: 199.

Nabours, R. K.
1917. "Studies of Inheritance and Evolution in Orthoptera, II," *Journ. Gen.*, 7: 1–47.
1925. "Studies of Inheritance and Evolution in Orthoptera, V," Kansas Agr. Exp. Station, *Technical Bulletin* 17.

Winge, O.
1927. "The Location of Eighteen Genes in *Lebistes reticulatus*," *Journ. Gen.*, 18: 1–43.

Wright, Sewall
1929. "Fisher's Theory of Dominance," AMER. NAT., 63: 274–279.

A CYTOLOGICAL AND A GENETICAL STUDY OF *PETUNIA*

II. Notes on Certain Phases of Sterility

In testing out various strains of *Petunia* in order to secure lines homozygous for flower color and shape,[1] we were at once confronted with the problem of sterility. Since 1919 the senior author has carried on studies, more or less as a side problem and as time would permit, in various aspects of self and other forms of sterility in *Petunia*. When tests which are being carried on in the field this summer are completed, we hope to publish the results thus far obtained with a detailed account of the studies to date. It is believed, however, that some unusual forms of infertility which we have met are worthy of an advance announcement. If similar conditions of sterility have been found in other plants, we should be very glad to learn of them. If already described in the literature of the subject, the papers have escaped our attention.

A. Self Sterility

We very early found it impossible under normal conditions, both in the greenhouse and in the field, to secure any seed capsules as the result of selfing a pink form which we had purchased of a nearby florist under the local name of Bar Harbor Beauty. We designated this line population 12 (P12), and have continued it, except as stated later, by means of cuttings. These clones were repeatedly selfed during the first three years, but with the exception of one capsule in the third year no fruit set, the pistils dying with the flowers.

During the academic year 1922–23, the author first named (Betty Watt Brooks) devoted some time to the study of sterility in P12. As a result of several hundred selfings under various conditions, this heretofore stubborn self sterility was apparently overcome. In a series of tests based on the age of the blossoms, 87 per cent. of the flowers selfed at the time of opening, and up to ten hours after opening set good-sized capsules which were to all appearances normal. It would seem from these results that the pollen had become capable of normal growth but only at a definite age and over a very short period. In more than two hundred selfings made at the same time as the others and on the same plants, but after the flowers had been open from twenty-four to

[1] Margaret C. Ferguson, "A Cytological and a Genetical Study of *Petunia*"—I. *Bull. Torrey Bot. Club*, 54: 657–664, fig. 1 and pls. 35, 36, January, 1928.

forty-eight hours, neither capsules nor seeds were formed. The results of the ten-hour and earlier pollinations were so contrary to all our previous experiences with P12 that we could not accept them without further confirmation. It was practically impossible to repeat these experiments at once, and the study lapsed for a time.

The second-named author (Lydia B. Walsh) chose as a senior research problem in 1925–26 the further study of sterility in P12. We wished not only to verify the results already obtained in this population, but also to carry the study on to the F_1 and later generations. To our surprise, it was found that the clones of the plants used in 1922–23 were now even more self fertile than were the plants at that date. Not only did we receive as high as 86 per cent. fertility when selfing flowers that had been open for ten hours or thereabouts, but 89.4 per cent. of the pollinations after the flowers had been open forty-eight hours resulted in normal full-sized capsules. As stated above, the earlier pollinations with flowers forty-eight hours old did not yield a single seed capsule.

All the F_1 plants derived from the now self fertile parents have been, since they first bloomed in 1926, normally self fertile. But the F_2 plants to which they gave rise in 1927 have to date produced no viable seed when selfed. F_2 plants are now being studied to determine whether they can by any means be rendered self fertile, or will they in time, like their grandparents, become self fertile with no known change in cultural conditions? Will the F_3 plants when secured be self fertile and the F_4 self sterile? Our studies to date indicate that P12 is a strain of *Petunia* which is unstable as regards self sterility or self fertility, although adhering very consistently to either state through a series of years. They further suggest that only alternating generations are self sterile when young.

It is of interest that these changes in self fertility and self sterility in this strain of *Petunia* are not confined to our experiments only. When in 1925 we again attempted to secure seeds or plants of the Bar Harbor Beauty, we found that the florist from whom we had purchased the original plants had given up growing this strain because he could get no seeds from his own plants and did not find them listed by other seed houses.

B. Cross Sterility

Petunia nyctaginiflora Juss. (P14) is very self fertile, setting when selfed large capsules often containing as many as nine hundred seeds. When this species is the female parent in crosses with

other strains, it also forms large capsules yielding, in all cases thus far tested, viable seeds. When at the same time and with the same plants crosses are made using P14 as the pollen parent, capsules do not mature and no seeds are formed. Only one exception to this has arisen. In a large number of crosses made by the second-named author with P14 as the pollen parent and a reddish purple strain of *Petunia*, which is known in our cultures as population 5 (P5), as the other parent, slightly over 2½ per cent. of the pollinations resulted in small capsules containing from twelve to thirty seeds.

That the pollen of this species is very active under certain conditions is evidenced by the fact that it produces unusually large capsules of viable seeds when self-pollinated. But we have still further evidence of its virility, in that it responds with full vigor when placed upon the stigmas of its own sibs. That is, when the pollen of P14 is placed upon the stigmas of the F_1 hybrids of which it was the female parent, normal capsules with viable seeds are formed. Furthermore, all the F_2 plants thus far tested have been equally fertile, when pollen from the grandmother generation was used. It is possible that the exception mentioned above may indicate a remote kinship between our P14 and P5, since the hybrids of *P. nyctaginiflora* Juss. and *P. violacea* Lindl. are recognized as the leading progenitors of our cultivated varieties of *Petunia*.[2] Has our P5 retained something from the nyctaginiflora line that has been lost by our other cultivated strains?

As a result of our studies to date on *Petunia nyctaginiflora* Juss., this species is found to be very self fertile and also to give rise to large hybrid generations when foreign pollen is used upon its stigmas. But while pollen from a given flower is active upon itself and its own sibs, it, with one possible exception, results in complete sterility on the stigmas of foreign or non-related strains.

BETTY WATT BROOKS
LYDIA B. WALSH
MARGARET C. FERGUSON

VARIATIONS IN *GONIONEMUS MURBACHII*

C. W. HARGITT[1] gives a summary of variations among the Hydromedusae, including the variations found among 2,000 specimens of *Gonionemus murbachii* collected in the Woods Hole region. During the present summer over 850 specimens of

[2] L. H. Bailey, "The Survival of the Unlike," p. 465–467, 1911.

[1] *Biol. Bul.*, 2: 221, 1900.

Gonionemus were collected and studied for further variations. Two rather striking variations were found which should be added to those listed by Dr. Hargitt.

The number of radial canals among the specimens examined by Dr. Hargitt ranged from two to six and there was a total of 4.82 per cent. deviation from the normal number of four. Deviation from the normal number of four radial canals among the 850 examined was 3.74 per cent., but this included an extreme variation not listed by Dr. Hargitt. One individual was found with seven radial canals, distributed evenly about the margin of the bell. The canals were not split at their proximal ends, as often

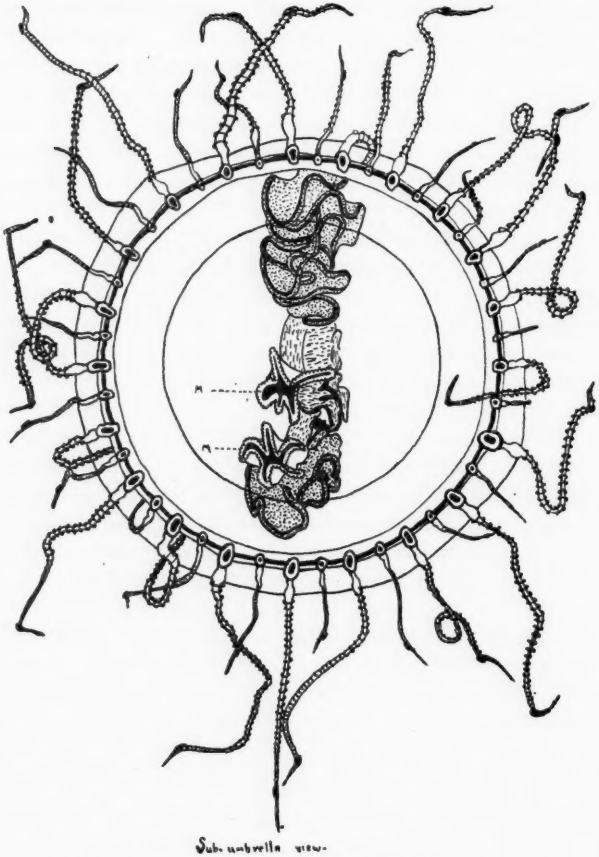

FIG. 1

occurs, but were perfectly normal radial canals. The individual was otherwise perfectly normal.

The other and more striking variation was an individual with two radial canals and two manubria. The only variations in manubria noted by Dr. Hargitt were slight variations in size and a spike-like projection from the side of the manubrium of a single individual.

The individual with two radial canals and two manubria is a female of approximately one centimeter in diameter. There are forty-two tentacles (which falls within the normal range of twenty-nine to seventy-two), and in the aquarium the individual acted quite normally in respect to food and tactile stimulation. Both manubria were functional and an attempt was made by the animal to engulf a small fish with both manubria at the same time. The gonads were rather full of eggs, although one of the gonads (and the adjacent radial canal) was somewhat shortened.

One manubrium was located as close to the center of the subumbrella surface as is common in Gonionemus and the other was located immediately adjacent to it. The musculature of each manubrium was apparently normal and there seemed to be no reduction in size of either. The gonad extending from the extra manubrium had lost that portion of its proximal folds which was displaced by the presence of the extra manubrium. This is true except for the slight increase in folding of this gonad under the velum, at its distal end.

Variations in themselves have little real significance, but when they have physiological or genetical involvements they are of genuine interest to biologists. There is room for further study on these variations, especially in light of their physiological effects and inheritance.

ROBERTS RUGH

WOODS HOLE, MASSACHUSETTS

VARIATIONS IN THE NUMBER OF COSTAL SHIELDS IN CARETTA

IN 1889 Boulenger[1] wrote: "The enormous amount of variation in the large series of loggerhead turtles in the [British] Museum leaves no alternative but to further multiply the number of species, or to admit only one." Among these variations are differences in the number of costal shields.

[1] Cat. Chel., Rhyn. and Croc., in the British Museum, 1889, p. 185.

The writer recently examined the loggerhead turtles in the collections of the British Museum (Natural History), the American Museum of Natural History, the Museum of Comparative Zoology and the Boston Society of Natural History—130 specimens—with the following results.

There were eighty-eight specimens of the Atlantic form. Eighty-one of these had the normal series of five pairs; seven were abnormal[2] as follows:

1	7 (left)	–6 (right)	Banana, Congo	(B. M.)
1	6	–6	" "	"
1	6	–5	Florida	(A. M. N. H.)
1	6	–5	"	"
1	5	–6	Cuba	"
1	6	–6	Florida	(M. C. Z.)
1	6	–6	"	"

There were thirty-two specimens from the Pacific and Indian Oceans. Three of these had the 5–5 formula of the Atlantic form. They were from Sharks Bay, Australia (B. M.). In the remaining twenty-nine specimens the series varied from 5–6 to 8–7, the largest group being twelve with six pairs each. There were five with seven pairs, the remaining specimens being asymmetrical.[3]

Thus it appears that the Atlantic form has normally at least five pairs of costal shields, fairly constant, while the Pacific and Indian Ocean forms may have six or seven pairs, possibly more, not always symmetrical.

By far the greater number of specimens in the collections mentioned are either very young or embryos. It is to be regretted that, in spite of the enormous number of adult sea-turtles captured for food and commercial purposes during the last hundred years, so few have been received by museums, for it is only from the study of large series of adult forms that definite conclusions as to classification can be reached.

HAROLD L. BABCOCK

BOSTON SOCIETY OF NATURAL HISTORY

[2] In a series of young and embryos of the Atlantic form from Beaufort, Coker found the small first costal wanting in seven instances. Symmetry in this abnormality was never noted, and in two of the seven specimens a supernumerary shield was present on the opposite side. One specimen showed a foreshortening of the body with only three pairs of costals. "Diversity of the Scutes of Chelonia," *Journ. Morph.*, Vol. 21, No. 1, 1910.

[3] Eschscholtz's drawing of the type of *Caretta olivacea* (from Manila Bay, P. I.) shows costals 6–7. The head is narrow. *Zool. Atlas*, Pt. 1, 1829, p. 2, pl. 3.